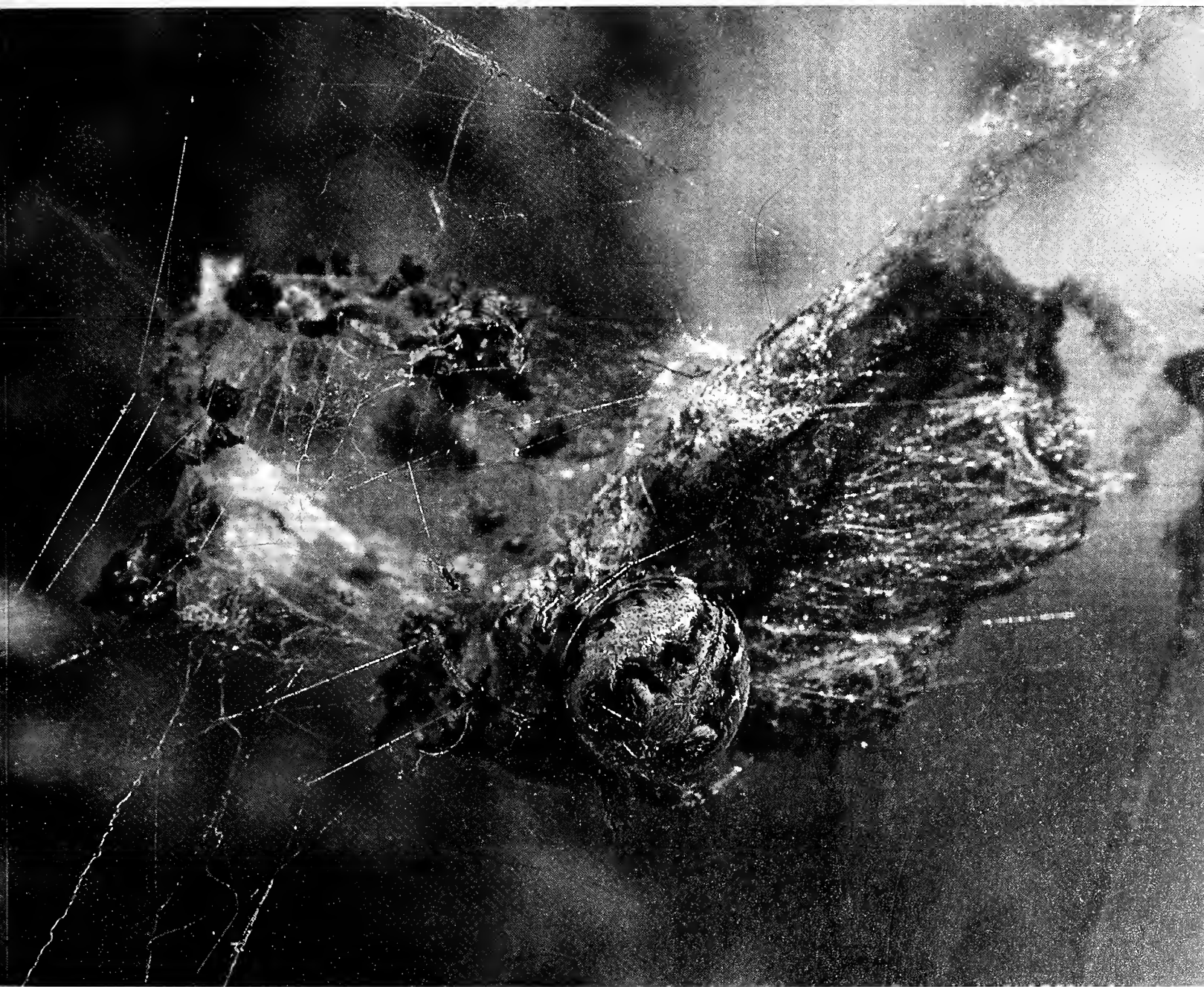


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## Editorial

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This issue of the JOURNAL OF RESEARCH ON THE LEPIDOPTERA is offered in a new format to permit greater efficiency in publication by using a docutech system. The system requires standard sized paper with most publications now in the 8.5 x 11 inches or A4 format.

On a per volume basis publication is now five volumes behind. This has been achieved by publishing single issue volumes since 1993 (volume 32). Note that the billing basis has been the equivalent of two volumes for one. Thus the price-per-page costs are the same as they were historically. This plan will continue until we will have material to publish two issues per volume and the volume will be equivalent to the year. We plan to issue single issue volumes until we catch up. Our schedule will be to publish as rapidly as suitable papers become available. We believe our capability is now limited only by manuscript receipt.

Volume 36 was sent to all members (and subscribers) without charge in 2000, anticipating a billing to include volume 37. This billing is now in the mail to you and includes the next two volumes, 38 and 39, which are in production.

The predominant reason for the delay in keeping the JOURNAL current has been lack of an established publication system: manuscript handling, review, typesetting, printing, mailing and, most critically, business management. These tasks have been largely family performed. With other priorities in our volatile world and economy these wholly volunteer tasks received low priority. The decision to reimburse key personnel should provide recognition of worth as well as modest incentive to now get on with the job of keeping this valuable intellectual resource professional.

Typesetting, printing, and mailing of the JOURNAL will be done in the Czech Republic where Dr. Zdenka Krenova will serve as technical manager. Editorial and business functions will remain in California under the temporary guidance of Dr. Rudi Mattoni. Billings will be made to both regular members and subscribers for each set of two volumes, irrespective of the time of issue. Thus a billing will be sent for this volume and volume 36 that was issued in the year 2000. The billing will additionally include volumes 38 and 39, volumes that we have started to prepare.

The LEPIDOPTERA RESEARCH FOUNDATION is in excellent financial condition. Funding is no constraint to publication. Page charges remain voluntary, except for color figures that we are still determining at this time.

With the universal availability of Internet communication, both inquiry and publication will be streamlined. We plan to virtually eliminate the use of regular mail except for sending the hardcopy JOURNAL itself. In order to efficiently communicate with our whole membership, we urge you to immediately send your e-mail address to the editor: (mattoni@ucla.edu). We will, of course, keep this information confidential in our files.

The objective of the JOURNAL will continue to emphasize scholastic work across all fields of scientific endeavor that use butterflies and moths. The publication will particularly aim to educate all levels of interest in these important animals and especially serve as a vehicle to encourage new generations of incipient biologists. The central focus for published work will remain evolutionary biology, ecology, conservation, and systematics. However, all relevant work will be considered.

Although the busy lives of most workers in the field make the demands of reviewing manuscripts today more difficult than ever, we intend to maintain the highest standards possible through the peer review process. Thoughtfully prepared and well written papers are the life blood of excellent science. We encourage all members to submit their research, both for the enormous self-satisfaction provided and to encourage others by example. Membership is not a criterion for publication when financial hardship is an issue. Workers without resources in less developed countries are welcome to submit suitable papers.

Over the past year, through the efforts of Dr. Carlo Mattoni, Dr. Zdenka Krenova, and Mr. Daniel Sojka Beran, we have scanned and PDF formatted all past issues of the JOURNAL OF RESEARCH ON THE LEPIDOPTERA. These will be available for anyone at our temporary website: [www.doylegroup.harvard.edu/~carlo/JRL/jrl.html](http://www.doylegroup.harvard.edu/~carlo/JRL/jrl.html).

Harvard University has generously provided this site for the time being. A permanent site will be established when necessary.

We strongly believe the information in our field should be freely available to all interested parties. We wish to emphasize that the free availability of past issues does not imply that a paid membership base is not necessary. Quite the contrary. Present membership does not cover costs, the difference being made up by our endowment investments. Your help in urging your institution and other scholastically concerned lepidopterists to subscribe is encouraged and essential.

Please continue to support the FOUNDATION and its efforts.

R.H.T. Mattoni, editor



$$aq_A^2 + bq_A + c = 0$$

where

$$a = D_A; b = -(D_A + D_B); c = D_B(D_A + D_B); q_B = q_A D_B / D_A$$



## 20-hydroxyecdysone induces apoptosis in the labial gland of *Manduca sexta*

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**Abstract:** The labial glands of *Manduca sexta* die over 5 days during larva to pupa metamorphosis. This cell death is presumably triggered by endocrine cues. The mechanisms by which steroid hormones induce apoptosis, however, are poorly understood. To investigate the role that the insect molting hormone, 20-hydroxyecdysone (20HE), plays in apoptosis of larval structures, we injected animals with exogenous 20HE. Since metamorphosing *Manduca* larvae have high titers of 20HE in the hemolymph, we surgically removed the prothoracic glands, which secrete 20HE, in order to create hormone-free abdomens. Labial glands from whole animals were used to establish the baseline levels of apoptosis during metamorphosis. Lysosomes play a critical role in the degradation of insect tissues (salivary gland, intersegmental muscle, and fat body) and mammalian tissues (mammary gland, prostate gland, and uterus). Apoptosis was monitored using acid phosphatase activity and histochemistry to monitor lysosomes and TUNEL to detect DNA fragmentation. Glands that were exposed to 20HE displayed an increase in the number of lysosomes, movement of lysosomes, and activation of acid phosphatase compared to controls. Glands from abdomens that received a single injection of hormone demonstrated levels of apoptosis that were comparable to glands from abdomens that were subjected to multiple 20HE injections. Our results suggest that 20HE triggers apoptosis of the labial glands and that its continuous administration is not necessary to kill the cells.

**Key words:** 20-hydroxyecdysone, apoptosis, lysosomes, *Manduca*, labial gland, TUNEL

**Abbreviations:** (20HE) 20-hydroxyecdysone, (TUNEL) terminal deoxynucleotidyl transferase mediated dUTP nick end-labeling, (AP) acid phosphatase.

### INTRODUCTION

Apoptosis is readily recognizable in insects as larval tissues degenerate in order to make way for adult tissues (Miller 1950; Finlayson 1956). Insect tissues provide a preparation that is apoptosis-enriched as well as ease of experimentation.

Metamorphosis is an instructive context in which to study apoptosis (Lockshin & Williams 1965a). One striking aspect of metamorphosis is the need for coordinated action by many tissues. For example, the epidermis, which produces the cuticle, and the nervous system, which produces the behavior the insect needs to extricate itself from the old cuticle,

must both be ready to molt at the same time, and any lack of coordination is likely to result in the death of the insect (Fahrbach 1997). The apoptosis of insect tissues during metamorphosis occurs at specific times in postembryonic life. The precise mechanism by which a cell is committed to and undergoes apoptosis remains unknown. The steroid hormone 20-hydroxyecdysone (20HE) regulates the timing of naturally occurring apoptosis in insects (Schwartz & Truman 1982; Truman & Schwartz 1984; Bennett & Truman 1985).

*Manduca sexta*, the tobacco hornworm, is an ideal model system for the study of apoptosis. Several tissues in *Manduca* undergo apoptosis during development. For example, at pupation the abdominal appendages used by the caterpillar for walking, the prolegs, disappear. This loss of peripheral structures is followed by the death of some of the motor neurons that terminated on the proleg muscles (Weeks & Truman 1985). The deaths of neurons at the end of larval life and adult abdominal ganglia after eclosion occur by apoptosis (Ewer et al. 1998). The prothoracic glands, which synthesize and secrete 20HE, initiate apoptosis during the pupa to adult metamorphosis (Dai & Gilbert 1997). *Manduca* muscles, like the dorsal external oblique 1 muscle, also die by apoptosis (Hegstrom et al. 1998). In many instances the death of these tissues can be blocked by protein synthesis inhibitors, suggesting that de novo protein synthesis is required (Weeks et al. 1993). The labial gland of the tobacco hornworm undergoes apoptosis during larval to pupal metamorphosis (Jochová et al. 1997a). This gland is much bigger and easier to isolate than the aforementioned *Manduca* tissues. The large size of the labial gland facilitates the execution of histochemical and microscopic techniques.

The labial gland (a homologue of the silk gland of *Bombyx mori* and the salivary gland of *Drosophila*) dies in 5 days during the larva to pupa transformation. The paired epithelial labial gland is a secretory gland that is approximately 0.2 mm in diameter, 17 cm long, and consists of a single layer of gigantic cells (100 x 100 x 40  $\mu$ m; Jochová et al. 1997a). The entire gland dies, except for the anterior duct, which differentiates into the labial gland in the adult moth. The labial gland provides a valuable system to study the mechanisms that regulate apoptosis since the death of the tissue is

synchronous and involves nearly the entire tissue. Consequently, a substantial amount of homogeneous dead cells can be studied uncontaminated by living cells, which is not possible in vertebrate systems. Since apoptosis in the labial gland occurs over 5 days, we can isolate glands at different stages of development and sequentially study differences in the levels of cell death. Previously, we have shown by metabolic measurements that the levels of energy resources and second messengers are adequate during the earlier phases of cell death in the labial gland (Halaby et al. 1994).

Lysosomal activation is an integral part of apoptosis in some systems, including insect tissues during metamorphosis and degenerating mammalian tissues. The salivary glands of the blowfly, *Calliphora vomitoria*, and *Drosophila* undergo a cell death that involves autophagic digestion by lysosomes (Bowen et al. 1996; Jones & Bowen 1993). Acid phosphatase has been used as the marker enzyme for lysosomes and a marker for apoptosis. Zakeri et al. (1994) demonstrated that the interdigital regions of normal mouse limbs displayed positive labeling for acid phosphatase by histochemistry. Acid phosphatase activity is augmented and lysosomes degrade the following tissues during apoptosis in mammals: mammary gland, prostate gland, ovary, and uterus (Helminen & Ericsson 1971; Searle et al. 1973; Verma 1983; Sensibar et al. 1990; Kasuya 1997). Here, we demonstrate that the movement of lysosomes, activation of lysosomal enzymes, and detection of single-stranded DNA breaks in dying labial gland cells are regulated by 20HE.

## MATERIALS & METHODS

### Rearing of Animals

*Manduca sexta* larvae were purchased from Carolina Biological Supply Company (Burlington, NC), grown in individual compartments at 25 °C with a 12 h photoperiod, and fed an artificial hornworm diet (Carolina). The larval to pupal metamorphosis was first detectable as the larvae underwent the initiation of wandering on day 0. This includes the cessation of feeding, exposing of the aorta, and seeking a place to burrow (Dominick & Truman 1985). Larvae were staged in terms of days prior to or after wandering.



## Ligations

At day 0, *Manduca* larvae have relatively high levels of endogenous 20HE in the hemolymph (Bollenbacher et al. 1981). Consequently, it is difficult to ascertain whether alterations in cell death parameters are due to endogenous or exogenous 20HE. To circumvent this problem, we created essentially 20HE-free abdomens. The relatively large size of *Manduca* full grown fifth instar larvae (approximately 12 g) facilitates surgical and endocrine manipulations. The only known sources of 20HE in *Manduca* are the prothoracic glands, which are located in the first thoracic segment. Day 0 larvae were anesthetized on ice for 20 min, ligated around the first abdominal segment, using dental floss, and the anterior body was severed to remove the prothoracic glands. The wounds were sealed with Krazy Glue (Borden, Columbus, OH). Isolated abdomens were left untouched overnight to allow sufficient time for the endogenous 20HE levels to decline.

## 20HE Injections

Abdomens received either a single injection or one injection every 24 h of either 50  $\mu$ g of 20HE or an equal volume of 10% ethanol (vehicle) prior to dissection of the labial glands. Each injection was placed in a different abdominal segment to avoid excessive damage to one site. Incubation times ranged from 0-120 h. The 20HE concentration in the isolated abdomens was between  $2.2$ - $2.9 \times 10^{-5}$  M, which are physiological concentrations of the hormone (Bollenbacher et al. 1981).

## Tissue Collection

Animals were anesthetized on ice for 10 min and the labial glands dissected. The anterior ducts, which do not undergo apoptosis, were not included in the experiments. Glands were fixed in 4% paraformaldehyde at 4°C overnight, frozen in Tissue Tek OCT (Miles, Elkhart, IN), and cut as 5  $\mu$ m sections onto poly-L-lysine coated slides (Sigma).

## Acid Phosphatase Assays

To visualize lysosomes and to monitor lysosomal enzyme activity we examined the marker enzyme of lysosomes, acid phosphatase (Pelletier & Novikoff 1972). Lysosomes were localized from slides of frozen sections using a histochemical acid phosphatase (AP) assay (Sigma, St. Louis, MO) as previously described (Halaby et al. 1994) with the exception

that all incubations were performed at room temperature to accommodate insect tissues. Labial glands from whole animals undergoing metamorphosis as well as from isolated abdomens were used. The presence of AP was indicated by red focal precipitates, which were resolved by light microscopy.

A biochemical AP assay (Sigma) was performed by homogenizing glands in 0.5 ml of 0.9% NaCl and clarifying homogenates by centrifugation for 5 min at room temperature. The reaction mixture (0.5 ml of p-nitrophenyl phosphate (substrate), 0.5 ml of 90 mM citrate buffer, pH 4.8, and 0.1 ml of homogenate) was incubated for 30 min at room temperature, and the reaction was terminated by the addition of 5 ml of 0.1 N NaOH. In alkali, liberated p-nitrophenol was measured spectrophotometrically at 410 nm. This assay was used to assess the lysosomal enzyme activity of labial glands that were obtained from intact and ligated animals.

## DNA Fragmentation

DNA fragmentation was assessed in frozen sections by a TUNEL method using the ApopTag (In Situ Apoptosis Detection Kit (Intergen,

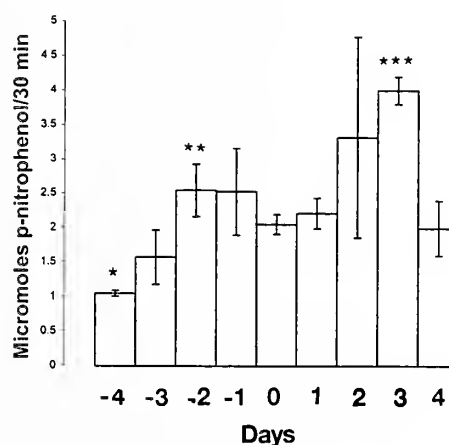


Fig 1. Acid Phosphatase Activity in Labial Glands during Metamorphosis. Labial glands were isolated from intact animals at various stages of development and the biochemical acid phosphatase (AP) assay was performed as described in Materials & Methods. The ages, representing days during the final larval stage, at dissection are indicated on the x-axis. Day 0 marks the beginning of larval to pupal metamorphosis. The values represent means of at least three independent experiments  $\pm$  SEM. Asterisks indicate values significantly different from day -3: \*,  $p < 0.02$ ; \*\*,  $p < 0.04$ ; and \*\*\*,  $p < 0.004$ . Student's *t*-test was used for determination of statistical significance. Total AP activity is expressed on the y-axis as micromoles of p-nitrophenol (the product of the reaction catalyzed by AP) released per 30 min.

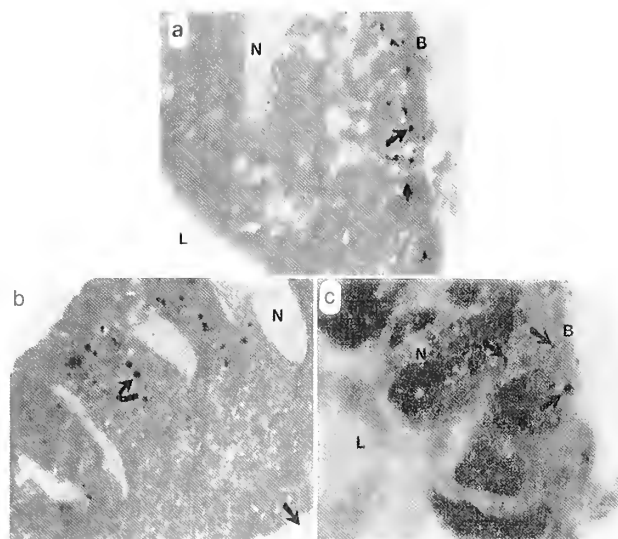


Fig. 2

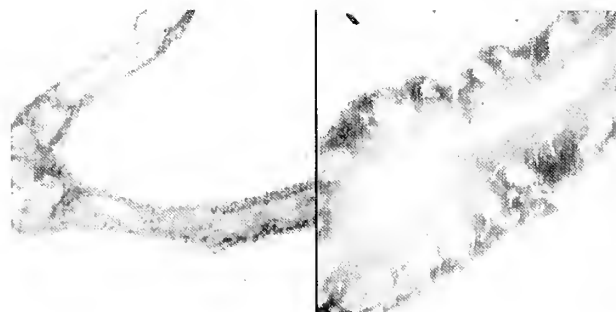


Fig. 4

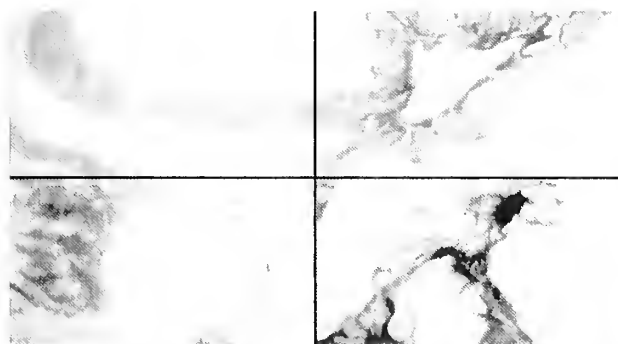


Fig. 5

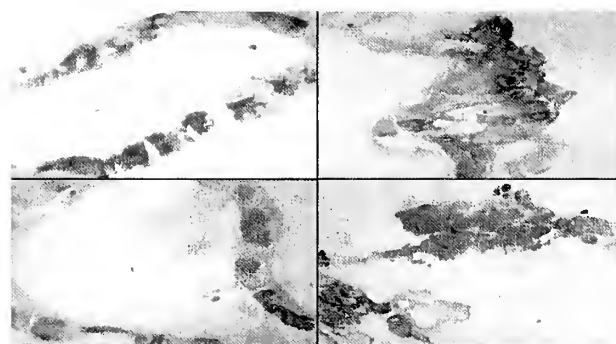


Fig. 6

Fig. 2. Histochemical Localization of Acid Phosphatase in Labial Glands during Metamorphosis. Glands were isolated from whole animals undergoing metamorphosis. (a) day -1 gland. (b) day 0 gland. (c) day 3 gland. Lysosomes were visualized using a histochemical AP assay. The presence of AP (arrows) is indicated by red focal precipitates (days -1 and 0) or diffuse staining (day 3), which were resolved by light microscopy. The lysosomes are restricted to basolateral regions of the cell on day -1. Lysosomes begin to migrate from basolateral areas towards apical, luminal regions on day 0. By day 3 the lysosomes have increased in number, are located throughout the cytoplasm, and have migrated into the lumen. Lumen (L). Nuclei (N) appear intact. Basolateral surfaces (B). Magnification: 1,000X. Microscope: compound.

Fig. 4. Effect of a Single Exposure of 20HE on Lysosomes. Animals were ligated at day 0. Isolated abdomens received a single injection of 20HE or vehicle and glands were processed for the histochemical AP assay after 120 h. 20HE increased the number of lysosomes (red stain; right panel) and cell death in experimental glands compared to control glands. This suggests that a single exposure of the glands to 20HE may be sufficient to trigger apoptosis. Magnification: 100X. Microscope: compound.

Fig. 5. DNA Fragmentation during Metamorphosis. Glands were isolated from day 0 (a) and day 4 (b) metamorphosing whole animals. DNA fragmentation was assessed by TUNEL as described in Materials & Methods. Weak TUNEL staining was detected at day 0 (a). Remnant nuclei persisted which displayed intense TUNEL staining on day 4 compared to day 0 glands. Nuclei (N). Magnification: 100X. Microscope: compound.

Fig. 6. Effect of 20HE on DNA Fragmentation. Abdomens were injected with 20HE or vehicle. Some abdomens received one injection (lower panels) while others received multiple injections (one every 24 h; upper panels). In either case glands were assayed at 120 h for DNA fragmentation by TUNEL. Arrows indicate positive labeling of DNA single-strand breaks. A single administration of 20HE (lower right panel) induced a level of DNA fragmentation that was comparable to glands that were exposed to multiple treatments of 20HE (upper right panel). 20HE-treated glands displayed fewer nuclei, however the TUNEL labeling of the remaining nuclei was more intense than that observed in control glands (left panels). Magnification: 100X. Microscope: compound.

Purchase, NY). To digest the sections, Oncor protein digesting enzyme (20 µg/mL) was applied to the specimens for 15 min at room temperature followed by four washes in distilled H<sub>2</sub>O for 2 min per wash. After application of equilibration buffer to the slides for 5 min, incubation with terminal deoxynucleotidyl transferase (TdT) and digoxigenin-11-dUTP was performed in a humidified chamber for 90 min at 37 °C, using plastic coverslips. Plastic coverslips were used to ensure even staining of the samples. The incubation was stopped by placing the slides in stop wash buffer for 30 min at 37 °C, in a Coplin jar. The slides were washed in 3 changes of phosphate buffered saline for 3 min each wash prior to being incubated with anti-digoxigenin peroxidase conjugated antibody, using plastic coverslips, in a humidified chamber for 30 min at room temperature. Slides were stained in diaminobenzidine (DAB; Research Genetics, Inc, Huntsville, AL) using coverslips for 2 min and counterstained with methylene blue (Sigma) for 1 min in a Coplin jar. The slides were mounted with Crystal/Mount (Biomedda, Foster City, CA). The brown DAB color product, which indicates staining of the free 3'-OH ends that occur as a result of DNA fragmentation, was observed by light microscopy.

## RESULTS

### Lysosomal Localization and Activity in Glands during Metamorphosis

We determined the localization of lysosomes and baseline levels of AP activity during metamorphosis in glands from whole animals. Glands were isolated from animals at various stages and biochemical values of AP were determined. AP activity started to rise as early as day -2, it leveled off, and peaked at day 3 (Fig. 1). Prior to day 1, the activity reflects primarily the growth of the gland. The histochemical AP data are in agreement with our biochemical AP results. Lysosomes from day -1 labial glands were restricted to basal regions of the cell (Fig. 2). By day 0, lysosomes had migrated from basolateral to luminal regions (Fig. 2). By day 3 the lysosomes increased in number and filled virtually the entire cytoplasm, and the gland finally disintegrates (Fig. 2). Day 3 represents the peak of apoptosis in the glands based on our biochemical and histochemical AP findings. These data suggest that alterations in lysosomes and lysosomal enzymes

are one of the earliest detectable changes that occur in degenerating glands.

### Effect of 20HE on Lysosomal Activity in the Labial Gland

Isolated abdomens from ligated day 0 animals were injected with one daily injection of either 20HE or vehicle for various periods. Exposure of labial glands to 20HE increased the levels of AP activity after 8 and 72 h incubations (Fig. 3). The increase at 8 h suggests that the 20HE injection mimics the first endogenous peak of the hormone on day -0.5 (Bollenbacher et al. 1981). The result at 72 h suggests that 20HE induces apoptosis after 3 days of multiple injections. This timeframe, 3 performed the following experiments. A single injection of 20HE or vehicle was administered to abdomens and apoptosis was assessed after 120 h. This incubation period was chosen because the gland dies over a five-day interval during metamorphosis. A single injection of 20HE triggered cell death as indicated by the increase in the number and movement of lysosomes after 120 h by histochemistry (Fig. 4).

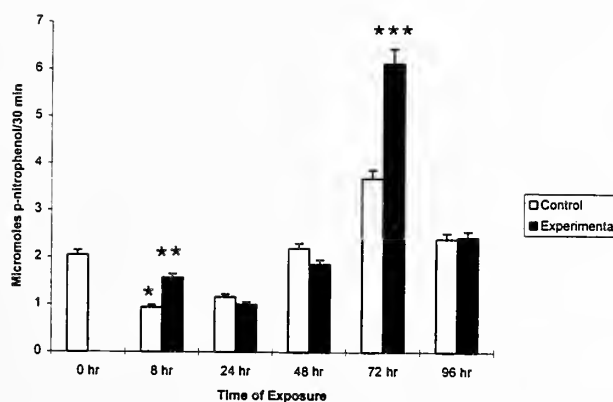


Fig. 3. Effect of 20HE on As Activity in Labial Glands from Abdomens. Animals were ligated at day 0 to remove the source of endogenous 20HE production. Abdomens were injected with 10% ethanol (vehicle; open bars) or 50 mg of 20HE (filled bars). One injection was given for the 8 h and 24 h incubation periods, while one injection every 24 h was administered at the other times. The biochemical As assay was performed on the glands. Values represent means  $\pm$  SEM of at least three independent experiments. 20HE induces apoptosis in labial glands after 72 h. Single asterisk indicates significant differences from 0 h,  $p < 0.03$ . Double and triple asterisks indicate significant differences between hormone treated and control glands: \*\*,  $p < 0.001$ ; and \*\*\*,  $p < 0.004$ .

### Effect of 20HE on DNA Fragmentation

To determine DNA fragmentation, we assessed the presence of single-strand breaks by TUNEL technique, as illustrated in Figures 5 & 6. Labial glands were isolated from intact metamorphosing animals as well as from isolated abdomens that were injected with vehicle or 20HE. TUNEL staining was barely detectable as the gland enters metamorphosis, day 0 (Fig. 5). An intense signal, however, was detectable at day 4 (Fig. 5). One set of isolated abdomens received multiple injections, one every 24 h, of vehicle or 20HE. The intensity of staining in nuclei from experimental glands was higher than that of controls at 120 h (Fig. 6). However, there were fewer nuclei in 20HE-treated glands. Glands that were exposed to a single 20HE treatment displayed similar TUNEL staining, as did glands that were exposed to multiple 20HE treatments (Fig. 6). In addition, 20HE-treated glands displayed a morphology that was similar to that of dying glands (Fig. 5).

### DISCUSSION

There is no doubt that 20HE regulates apoptosis in a variety of insect tissues. However, the precise mechanisms by which the hormone directly or indirectly induces cells to undergo cell death are not yet understood. Like other steroid hormones, 20HE when bound to its receptor, acts as a transcriptional activator. Indeed 20HE regulates several genes that are involved in apoptosis. The three pro-apoptotic genes so far cloned in *Drosophila*, reaper, head involution defective (*hid*), and grim, are all upregulated by 20HE (White et al. 1994; Grether et al. 1995; Vucic et al. 1997). The upregulation of reaper and *hid* mRNAs immediately precedes the destruction of the larval salivary glands in *Drosophila* (Dorstyn et al. 1999). These are very encouraging data because these gene products may exist in *Manduca* tissues as well. 20HE should also activate death genes that are responsible for the demise of the labial glands.

The movement of lysosomes from basolateral to apical regions in the cells of the labial gland (Halaby et al. 1994; and this report) suggests that lysosomes play a pivotal role in the destruction of the cell. The lysosomal movement, presumably a result of alterations in cytoskeletal components, specifically

microtubules, is currently under investigation. The cytoskeleton has been shown to undergo reorganization during the death of the salivary gland in *Drosophila* (Jochová et al. 1997b). The increase in lysosomal enzyme activity during labial gland degeneration is one of the earliest detectable morphological markers of apoptosis (Zakeri et al. 1993; Halaby et al. 1994). 20HE may directly stimulate acid phosphatase activity in the labial glands as was shown to be the case in *Corcyra cephalonica* (Ashok & Dutta-Gupta 1988). Lysosomal hydrolases are prominent during the histolysis of insect intersegmental muscles (Lockshin & Williams 1965a,b) and salivary glands (Aidells et al. 1971). Lysosomal enzymes also play pivotal roles in the apoptotic deaths of the mammary gland, prostate gland, and uterus (Helminen & Ericsson 1971; Moulton & Koenig 1983; Sensibar et al. 1990). The selective activation of these hydrolases may be used therapeutically, such as their employment to induce tumor regression of mammary carcinomas (Gullino & Lanzerotti 1972; Cutts 1973), as a means of killing harmful cells while sparing healthy ones.

The DNA fragmentation induced by 20HE in labial gland nuclei was detected later than were the early lysosomal-induced cytoplasmic damage. This pattern of cell death has also been observed in *Calliphora* salivary gland (Bowen et al. 1993) and mammalian mammary gland (Strange et al. 1992; Tenniswood et al. 1992; Zakeri et al. 1995). Our results indicate that 20HE may promote accelerated apoptosis in the labial gland, but that the TUNEL technique may not be sensitive enough to detect this DNA destruction (Labat-Moleur et al. 1998; Cuello-Carrion & Ciocca 1999). This is presumptively due to the fact that the nuclei in hormone-treated glands are being preferentially degraded, resulting in fewer free 3'-OH ends available for the TdT-catalyzed reaction to occur. Exogenously administered 20HE has been demonstrated to promote the accelerated demise of nuclei from muscle in *Manduca* moths (Hegstrom & Truman 1996; Hegstrom et al. 1998) and nuclei from *Manduca* larval muscles and motoneurons (Weeks & Truman 1986).

Our results indicate that a single injection of 20HE can trigger complete cell death of the labial gland (Figs. 4 & 6). The single exposure of the labial glands to the hormone was sufficient to induce steroid-triggered apoptotic responses, DNA fragmentation and activation of lysosomes.

Others have reported, using in vitro experiments, that a brief rather than continuous exposure of insect organs to 20HE resulted in apoptosis of those tissues (Jiang et al. 1997; Streichert et al. 1997). Further research is needed to elucidate the exact mechanism by which 20HE and other steroid hormones cause apoptosis.

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# The butterflies of Jordan

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**Abstract.** A total of 63 species of butterflies are recorded from the different ecological zones in Jordan as a result of the examination of more than 3350 specimens in the collection of the University of Jordan Insects Museum and the Natural History Museum at Yarmuk University. These specimens were collected from different parts of Jordan from 1974 to 1999. *Colotis danae* and *Anthocharis gruneri* (Pieridae) are recorded for the first time. Collecting sites, seasonal occurrence and available biological or ecological notes are given for each species. An updated list of the butterflies of Jordan is given based on this study and previous records, bringing the total to 91 species and subspecies.

**Keywords:** Butterflies, Rhopalocera, Jordan.

## INTRODUCTION

The butterflies of Jordan have been studied since the beginning of this century. Graves (1925) studied the collection acquired by Mr. Philby while serving in Jordan. Hemming (1932) gave the first comprehensive study on the Jordanian butterflies. A series of papers were published by Larsen (1975, 1976, 1977, 1984 a, b) about the butterflies of Jordan and nearby areas. Al Musa (1979) listed 40 species of butterflies and 69 species of moths from Jordan. However, the comprehensive study of Larsen & Nakamura (1983) is still our main reference, which was based on several collections either housed in Jordan or abroad. Bozano (1990) recorded 52 species based on two visits to the country in the spring of 1989. Ten Hagen (1995, 1996) reported on the butterflies of Syria and Jordan. Amr et al. (1997) reported on 11 species from Al Azraq Reserve in the eastern desert. Fabiano (1998) conducted several visits to the southern desert of Jordan in the spring months of

1992 to 1996 and recorded 35 species.

Jordan is the southernmost outpost of many Palaearctic species and a northern frontier for several tropical and eremic butterflies (Larsen & Nakamura, 1983). Although Jordan is a small country, the presence of several phytogeographical zones makes the Jordanian butterfly fauna interesting. However, Jordan has undergone drastic ecological changes in the form of agricultural development coupled with extensive use of pesticides, urbanization and destruction of natural habitats, which certainly affected species composition and distribution of the butterflies of Jordan.

The aim of this paper is to present an update on the butterflies of Jordan based on the examination of a large series of butterflies housed in the University of Jordan Insects Museum, the Natural History Museum at Yarmouk University, and on records in previous literature. We also included our field observations during field trips conducted in different parts of Jordan during the last several years.

## MATERIALS AND METHODS

Butterfly collecting in all biotopes of Jordan was conducted from 1993 to 1999. In addition, specimens housed at the University of Jordan Insects Museum and the Jordan Natural History Museum, Yarmouk University were examined. We studied also a small collection from the newly established Al Mujib Nature Reserve, which was collected by Paul Hendig, a volunteer with the American Peace Corps working with the Royal Society for the Conservation of Nature. Common names given at the remarks section follow Higgins & Riley (1970), Larsen (1983) and Walker & Pittaway (1987). The geographic names for localities are arranged alphabetically and follow the Gazetteer of Jordan (Anon 1990). Data about global distribution, previous records, phytogeographical zones, and hosts of species follow mainly Larsen & Nakamura (1983). The number of specimens examined is given for each species. Numbers of specimens collected in each month is presented between brackets in order to give an approximate idea about the seasonal occurrence.

We have downgraded two subspecies in accordance with the recent review of Turkish butterflies by Hesselbarth et al. (1995); these changes are endorsed by Larsen (pers. comm.).

## RESULTS

### *Papilionidae*

#### *Papilioninae*

##### *Papilio machaon syriacus* Verity, 1905

*Material.* 41 specimens. *Localities.* Al Aridah road, Al Hammah, Al Jubayhah, Ar Rusayfah, As Salt, Ghawr Kabid, Jarash, Ma'in, Natifah, Na'ur, Wadi al Arab, Zayy.

*Collecting months.* MAR (3), APR (12), MAY (10), JUN (4), JUL (4), AUG (0), SEP (0), OCT (5), NOV (3).

*Remarks.* The Swallowtail is a Holarctic species with a wide range of distribution, but the subspecies *syriacus* is confined to the Levant and eastern Saudi Arabia (Pittaway et al. 1994). In Jordan, it occurs in the northern and southern Mediterranean zones and in the Jordan Valley. Larsen & Nakamura (1983) reported specimens as far south as Ras el Naqb. Collecting months suggests two broods, one

in early March and extends to May, followed by another in October. Larvae feed on several species of the families Apiaceae and Rutaceae. This beautiful butterfly is not common and its collection by the locals as an ornamental item may affect its population.

##### *Papilio alexanor maccabaeus* Staudinger, 1891

*Material.* 2 specimens. *Locality.* Al Jubayhah.

*Collecting months.* MAR (1), APR (1).

*Remarks.* The Tiger Swallowtail occurs in southern France, Italy, the Balkans, Asia Minor, Iran, Iraq, Turkestan, Afghanistan and north-western India. It is a rare species found in the northern Mediterranean zone of Jordan. Previously collected from Wadi Kufringi and Wadi Zarqa (Larsen & Nakamura 1983). Ten Hagen (1995) collected it from Na'ur. Al-Jubayhah represents the 4th locality for this rare species. Nakamura & Ae (1977) gave a comprehensive account on its biology, in which they indicated that peak activity occurs during April.

### *Zerynthiinae*

##### *Allancastria deyrollei eisneri* Bernardi, 1971

*Material.* 37 specimens. *Localities.* Al Aridah, Al Jubayhah, Amman, Ar Rumman, As Salt, Ash Shajarah, Ayn Abdah, Ayn Aqraba, Ghawr Kabid, Jarash, Madaba, Mahis, Sakib, Wadi Shu'ayb.

*Collecting months.* MAR (8), APR (25), MAY (4).

*Remarks.* The Lebanese Festoon is found in Turkey and the Levant. It is confined to the Mediterranean regions of Jordan, and less frequent in the Jordan Valley. Previously collected as far south as Petra in southern Jordan by Lockhart (Larsen & Nakamura 1983). Peak activity occurs during April in a single brood and declines thereafter. This species is associated with the Moorish Birthwort, *Aristolochia maurorum*, a mountainous plant of the family Aristolochiaceae. *Allancastria cerisyi speciosa* Stichel occurs in coastal areas of Palestine and Lebanon, but it appears that it can not penetrate into Jordan (Larsen & Nakamura 1983).

### *Parnassiinae*

##### *Archon apollinus* Herbst, 1798

*Material.* 103 specimens. *Localities.* Al Bunayyat, Al Ghawr, Al Jubayhah, Al Kufrayn, Al Muqabalayn, Amman, As Salt, Ash Shajarah, At Turrah, Ayn



Abdah, Dibbin, Ghawr Kabid, Irbid, Jarash, Madaba, Umm Qays, Yarqa.

*Collecting months.* FEB (2), MAR (52), APR (43), MAY (4), JUN (1), JUL (0), AUG (1).

*Remarks.* The False Apollo is a Pontomediterranean butterfly, limited to Bulgaria, Turkey, the Levant and Iraq. It is found in the Mediterranean zones and the Jordan Valley. Collecting months suggests one brood annually that occurs in March and April. Similar to *A. d. eisneri*, larvae prefer the Moorish Birthwort as a food source.

## Pieridae

### Pierinae

#### *Aporia crataegi augustior* Graves, 1925

*Material.* 96 specimens. *Localities.* Al Bunayyat, Al Fuhays, Al Jubayhah, Al Muqabalayn, Amman, Ar Rumman, As Salt, Jarash, Kufrinigah, Mursi, Northern Jordan Valley, Shunat Nimrin, Tila' al Ali, Umm kharrubah.

*Collecting months.* FEB (1), MAR (0), APR (85), MAY (10).

*Remarks.* The Black-veined White is widely distributed in the Palaearctic region. In Jordan, it is common in the mountainous areas and along the Jordan Valley. Peak activity occurs in April. Larsen (1977) reported on the seasonal fluctuation of this butterfly, and indicated that it becomes very scarce and later reappear in relatively high numbers. He reported that this phenomenon is known among populations occurring at the limit of its distribution. Larvae feed on *Crataegus*, *Prunus duleis* (Almond) and other Rosaceae where they may become pests.

#### *Pieris brassicae* Linnaeus, 1758

*Material.* 69 specimens. *Localities.* Al Hashimiyah, Al Jubayhah, Al Yadudah, Amman, As salt, Az Zarqa', Irbid, Joradn Valley, Madaba, Mashari', Sahab, Suwaylih, Wadi as Sir, Wadi Shu'ayb, Zayy.

*Collecting months.* FEB (18), MAR (8), APR (10), MAY (3), JUN (2), JUL (1), AUG (15), SEP (4), OCT (7), NOV (1).

*Remarks.* The Large White is found from North Africa via most of Europe and the Middle East to the Himalayas. It occurs in Chile and South Africa as an introduction. It is a migrant species common in the Mediterranean zones of Jordan from which it penetrates the Jordan Valley. Its presence

throughout of the year suggests that it have several broods. It feeds on several species of family Brassicaceae and *Capparis spinosa* (Capparidaceae). The subspecies *cataleuca* was believed to be an east Mediterranean subspecies is considered an ecological form.

#### *Pieris rapae leucosoma* Schawerda, 1905

*Material.* 202 specimens. *Localities.* Ajlun, Al Aridah, Al Baq'ah, Al Fuhays, Al Ghawr, Al Jubayhah, Al Kufrayn, Al Kirbah As Samrah, Al Mujib Nature Reserve, Al Mushqqar, Al Muwaqqar, Amman, As Salt, As Simakiyah, As Sukhnah, Az Zarqa, Dayr Alla, Dhulayl, Huwayrah, Irbid, Jarash, Kurayyimah, Madaba, Mahis, North Shunah, Suwaylih, Tila' al Ali, Umm Qays, Wadi Shu'ayb, Yajuz.

*Collecting months.* JAN (5), FEB (8), MAR (30), APR (44), MAY (12), JUN (15), JUL (8), AUG (7), SEP (13), OCT (34), NOV (24), DEC (2).

*Remarks.* The Small White is a migratory butterfly found throughout the Palaearctic region and as an introduction in North America, Australia and New Zealand. The subspecies *leucosoma* is mostly associated with the Mediterranean ecozone and the Jordan Valley. It was collected all-year round, and populations in the Jordan valley have several broods. Butterflies were observed in remote areas as Qasr Burqu' in the eastern desert as well as in the busy streets downtown in Amman. Amr et al. (1997) reported that it was one of the most common species found in Al Azraq Reserve.

#### *Pontia edusa* Fabricius, 1777

*Material.* 208 specimens. *Localities.* Abu Alandah, Ajlun, Al Aridah, Al Dhulayl Station, Al Fuhays, Al Ghawr, Al Hammah, Al Jubayhah, Al Mujib Nature Reserve, Al Mushaqkar, Al Wala, Al Lajjun, Amman, Ash Shawbak, As Salt, As Simakiyah, At Turrah, Ayn Qantara, Ayn at Turab, Az Zarqa', Bayt Yafa, Dayr Alla, Ghawr Kabid, Irbid, Jarash road, King Talal Dam, Kufrinijah, Mughaiyyir (Essirhan?), North Shunah, Rasun, Sadd el khaledyyeh, Sakib, Shafa Badran, Suwaylih, Tabarbawr, Tila' al Ali, Umm Qays, Umm Kharrubah, Wadi al Arab, Wadi al Yabis, Waqqas, Zayy.

*Collecting months:* JAN (2), FEB (7), MAR (7), APR (24), MAY (31), JUN (21), JUL (14), AUG (27), SEP (27), OCT (20), NOV (24), DEC (4).

*Remarks.* The Eastern Bath White occurs in the Sahara, most of Europe, India, Central Asia and east

Asia. It is one of the most common species inhabiting almost all parts of Jordan except the southern desert. It mainly feeds on a species of the genus *Reseda* (Larsen & Nakamura 1983). In Iraq, larvae attack mustard and other Brassicaceae (Al Hussein 1984). Amr et al. (1997) found it associated with areas of *Tamarix* and *Alhaji maurorum* in Al Azraq Reserve.

***Pontia glauconome glauconome* Klug, 1829**

*Material.* 2 specimens. *Locality.* Al Mujib Nature Reserve.

*Collecting month.* MAY (2).

*Remarks.* The Desert White is an eremic species found in North Africa, Arabian and Middle Eastern deserts. It occurs in the southern Jordan Valley, northern and southern deserts of Jordan. It is very scarce. It was collected from March to May. The pupa can diapause for several years. Larvae feed on *Zilla spinosa* L. (Brassicaceae) and *Ochradenus baccatus* Del. (Resedaceae). Amr et al. (1997) mentioned that it was a rare species in Al Azraq Reserve.

***Madais fausta fausta* Olivier, 1804**

*Material.* 167 specimens. *Localities.* Al A'al, Al Baqurah, Al Jubayhah, Al Mujib Nature Reserve, Amman, As Salt, Ayn al Bayda, Ayn at Turab, Az Zarqa', Dayr Alla, Ghawr As Safi, Irbid, Kharjah, Kufrinijah, Ma'in, Near Dead sea, North al Adasiyah, Rahub, Sadd al Khaldeyyeh, Shunat Nimrin, Wadi Shu'ayb, Wadi ar Rayyah, Zayy.

*Collecting months.* APR (2), MAY (0), JUN (3), JUL (5), AUG (91), SEP (35), OCT (21), NOV (9), DEC (1),

*Remarks.* This subspecies is found in north-western Egypt, the Middle East, the Arabian Peninsula, Iraq and south-western Iran. The Salmon Capar butterfly is a rather migratory species with a distribution confined to the Jordan Valley and the upper Mediterranean zone. Other earlier localities include Zarqa Main and Petra (Larsen & Nakamura, 1983). It seems that it has two broods, one in spring and another towards the end of July. Larsen (1975) suggested that a regular migratory contact with the Arabian populations occurs to ensure the survival of the Jordanian populations.

***Colotis phisadia phisadia* Godart, 1819**

*Material.* 4 specimens. *Localities.* Ghor as Safi, Ghor Khuneizierreh.

*Collecting months.* MAR (3), DEC (1)

*Remarks.* The Blue Spotted Arab is common in tropical Africa, Arabia and Jordan. It is limited to the Dead Sea area in Jordan but it is possible to have contacts with the Arabian populations through Wadi Arabah and Aqaba. It is a tropical element of the Jordanian fauna. The larval food plant is *Salvadora persica*.

***Colotis danae eupompe* Klug, 1829**

*Material.* 1 specimen. *Locality.* Mahis.

*Collecting month.* JUN (1).

*Remarks.* The Scarlet Tip is an Afrotropical butterfly being one of the most widespread butterflies in dry tropical Africa, but also occurs in India. Only one specimen is known from Egypt. It is common in southwestern Arabia and Dhofar. This species is recorded from Jordan for the first time and it is apparently very rare. The single specimen was collected on the 7th of June 1991. Larvae feed on *Cadaba* spp. and perhaps other Capparidaceae (Larsen 1990).

***Belenois aurota aurota* Fabricius, 1793**

*Material.* 22 specimens. *Localities.* Al Jubayhah, Al Mujib Nature Reserve, Amman, Ghawr Kabid, Ghawr as Safi, Nahlah, Shunat Nimrin, Wadi as Sir.

*Collecting months.* MAY (1), JUN (0), JUL (1), AUG (3), SEP (2), OCT (3), NOV (12).

*Remarks.* The Capar White is a strong tropical migrant butterfly. Larsen & Nakamura (1983) referred to several occasions citing the migratory behavior of this butterfly in Lebanon and Palestine. It prefers the warm Jordan Valley, however, it was collected from two localities within the eastern mountains. It feeds on *Capparis spinosa*.

***Euchloe ausonia melisande* Fruhstorfer, 1908**

*Material.* 50 specimens. *Localities.* Al Aridah, Al Jubayhah, Al Walah, Amman, As Salt, Dayr Abi Sa'ed, Dayr Alla, Gawr Kabid, Umm ar Rumman,

*Collecting months.* FEB (1), MAR (21), APR (25), MAY (2), OCT (1).

*Remarks.* The *E. ausonia* complex is found all around the Mediterranean and in Asia Minor. The Dappled White is common in both Mediterranean zones of Jordan. It feeds on *Brassica* and *Sinapis* (Brassicaceae).

***Euchloe belemia* Esper, 1799**

*Material.* 50 specimens. *Localities.* Al Jubayhah,

Al Aridah, Ash Shajarah, Ayn Abdah, Ayn Aqraba, Ayn Ghazal, Bayt Yafa, Dayr Alla, Ghawr Kabid, Irbid, Al Qarn, North Shunah, Qashab, Wadi al Arab, Wadi Shu'ayb.

*Collecting months.* FEB (7), MAR (20), APR (23).

*Remarks.* The Green-striped White extends from Iberian Peninsula, via North Africa to the Middle East and Iran to Baluchistan. In addition, it was recorded from Ethiopia and Arabia. It is a common species in the northern Mediterranean zone of Jordan and known to occur in the Jordan Valley. Apparently, it has one brood in the spring, with highest peak of emergence in April. It feeds on *Erucaria* in the Jordan Valley (Trought in Larsen & Nakamura 1983).

#### ***Euchloe charltonia* Donzel, 1842**

*Material.* 5 specimens. *Localities.* Al Karak, Azraq, Ghawr Kabid, Al Quarn, Wadi al Arab.

*Collecting months.* MAR (3), APR (1), MAY (1).

*Remarks.* The Greenish Black-tip is an eremic butterfly, distributed from North Africa via the Middle East to Afghanistan. In Jordan, it is mostly associated with the Irano-Turanian ecozone, with fewer populations occurring in the Jordan Valley. Amr et al. (1997) reported on its rare presence in Al Azraq Reserve. Previous collecting dates suggest up to three broods per year. It feeds on several species of *Diplotaxis*, and Rough and Sweet Stock (*Matthiola* sp.). Ten Hagen (1996) recorded *Euchloe penia* Freyer 1851 from Syria. It is possible that this species may occur in north Jordan, however, this needs further investigation.

#### ***Zegris eupheme uarda* Hemming, 1929**

*Material.* 3 specimens. *Locality.* Wadi Al Walah.

*Collecting month.* MAR (3).

*Remarks.* The Sooty Orange Tip occurs in dry parts of Spain and Morocco, the Dead Sea area, the desert between Jordan and Iraq, parts of Turkey and Iran, to dry Central Asia. In Jordan, The subspecies *urda* is limited to the Irano-Turanian zone separating the Mediterranean vegetation from the lower parts of Jordan Valley. One brood appears from late February to early April. The larvae feed on *Erucaria boveana* in Palestine. Pittaway (1985) described *Zegris eupheme larseni* from Saudi Arabia and Jordan. One paratype female was collected from Wadi Rum (south Jordan) by Larsen in 1977. Photographs of both male and female are given by Larsen (1983),

Bozano (1990) and Fabiano (1998).

#### ***Anthocharis gruneri gruneri* Herrich-Schäffer, 1851**

*Material.* 2 specimens. *Localities.* Ayn Aqrabah, Wadi As Salt.

*Collecting months.* FEB (1), MAR (1).

*Remarks.* The Grüner's Orange Tip is found in south Europe and Turkey (Higgins and Riley, 1970) and in Palestine (Larsen & Nakamura 1983). This species is recorded from Jordan for the first time. As Larsen & Nakamura predicted, this species is now recorded from the northern Mediterranean zone. The specimen from Ayn Aqraba was collected in 1993 while the other one in 1999.

#### ***Anthocharis cardamines phoenissa* von Kalchberg, 1894**

*Material.* 9 specimens. *Localities.* Ayn Abdah, Ayn Aqraba, Wadi al Arab.

*Collecting months.* FEB (1), MAR (8)

*Remarks.* The Orange Tip is found from western Europe, temperate Asia to Japan. Larsen & Nakamura (1983) included this species based on Trevor Trought's field notes. Our specimens confirm the presence of this species in Jordan. The localities indicated above are within the most north western part of the northern Mediterranean zone. This species is quite common in Lebanon and Palestine.

#### ***Coliadinae***

#### ***Colias crocea crocea* Geoffroy, 1785**

*Material.* 227 specimens. *Localities.* Ajlun, Dhulayl, Al Azraq, Al Ghawr, Al Jubayhah, Al Karamah, Al Kurayyimah, Al Mafraq, Amman, Ar Rumaymin, As Salt, Ash Shawbak, Ayn Qantara, Dayr abu Sa'id, Dayr Alla, Dead Sea area, Ghawr as Safi, Ibbin, Irbid, Jarash, King Talal Dam, Madaba, Nahlah, Qasr Al Hallabat, Qwaylibah, Sahab, Shunat Nimrin, Tila' al Ali, Umm Qays, Wadi as Sir, Wadi al Arab, Wadi Shu'ayb, Zayy.

*Collecting months.* FEB (1), MAR (4), APR (21), MAY (21), JUN (21), JUL (44), AUG (6), SEP (14), OCT (78), NOV (12), DEC (5).

*Remarks.* The Clouded Yellow is common in North Africa, Europe and the Middle East. In Jordan, it is common throughout the Mediterranean and the Irano-Turanian zones. Collecting dates suggest that it has several broods that fly all-year round. It feeds on several species of *Vicia*. Amr et

al. (1997) found it common near cultivated alfalfa (*Medicago sativa*) in Al Azraq Reserve.

***Gonepteryx cleopatra taurica* Staudinger, 1881**

*Material.* 3 specimens. *Locality.* Ajlun.

*Collecting month.* MAY (3)

*Remarks.* The Cleopatra is a typical Holomediterranean species. Although Larsen & Nakamura (1983) gave several localities within the northern Mediterranean zone, we have one single locality in northern Jordan. This is a forest-adapted species. Decline in its numbers and distribution may reflect the degradation of forests in Jordan. The larval food plants are *Rhamnus* spp.

## Nymphalidae

### Danainae

***Danaus chrysippus chrysippus* Linnaeus, 1758**

*Material.* 99 specimens. *Localities.* Al Jubayhah, Al Karamah, Al Kufrayn, Al Mu'addi, Al Mujib Nature Reserve, As Salt, Azraq, Dayr Alla, Ghawr as Safi, Ghawr Kabid, Jarash, Jordan River (at Prince Mohamad farm, Waqqas, and Zoor Baurah).

*Collecting months.* JAN (9), FEB (0), MAR (0), APR (3), MAY (6), JUN (0), JUL (27), AUG (7), SEP (0), OCT (15), NOV (10), DEC (24).

*Remarks.* The Plain Tiger is a migrant butterfly widely distributed in the old world tropics. It is common in the Jordan Valley, however, few specimens were caught from Azraq in the Eastern Desert and the Mediterranean region as well. It was seen migrating northward by the Jordan River in 1996 at Al baqurah in the extreme north west of Jordan. The main food plant is *Calotropis procera*, but other Asclepiadaceae are acceptable.

### Charaxinae

***Charaxes jasius jasius* Linnaeus 1767**

*Material.* 1 specimen. *Locality.* Rasun.

*Collecting month.* July (1).

*Remarks.* The Two-Tailed Pasha is the only Palaearctic off-shoot of the tropical genus, being local and uncommon species in the Middle East. It is a very rare species, only one specimen was collected from Rasun in the northern Mediterranean zone. The food plant is *Arbutus unedo*.

### Nymphalinae

***Junonia orithya* here Lang, 1884**

*Material.* 1 specimen. *Locality.* Al Jubayhah.

*Collecting month.* MAY (1).

*Remarks.* The Blue Pansy is a tropical migrant but the subspecies *here* is found in Arabia (Larsen, 1990). Larsen caught one specimen in the autumn of 1983 (Larsen 1984b). Benyamini (1990) indicated its occurrence on the western side of the Jordan Valley north of the Dead Sea. It was seen feeding on tiny white flowers of *Heliotropium bacciferum* in Saudi Arabia.

***Limenitis reducta schiffmuelleri* Higgins, 1933**

*Material.* 5 specimens. *Locality.* Rasun.

*Collecting months.* MAY (3), JUN (0), JUL (2).

*Remarks.* The Southern White Admiral is found in southern and central Europe to Iran. It is a rare species in Jordan. Larsen & Nakamura (1983) mentioned that only two records of this species were known from Jordan (Dibbin and Jarash). Rasun represents a third locality. All of these localities are in the northern Mediterranean zone to which the species appears to be limited. It feeds on *Lonicera* sp.

***Vanessa atalanta* Linnaeus, 1758**

*Material.* 7 specimens. *Localities.* Ajlun, Al Jubayhah, Ghawr Kabid, As Salt, Tabarbawr.

*Collecting months.* APR (1), MAY (1), JUN (1), JUL (0), AUG (0), SEP (0), OCT (0), NOV (3), DEC (1).

*Remarks.* The Red Admiral is migrant species that occurs in the Holarctic region. It is a scarce species in Jordan, mostly recorded from the northern Mediterranean zone but may be found in the Jordan Valley. The food plant is *Parietaria* and *Urtica pilulifera*.

***Vanessa cardui cardui* Linnaeus, 1758**

*Material.* 198 specimens. *Localities.* Abu Alandah, Ajlun, Al Aridah, At Turrah, Al Jubayhah, Al Kufrayn, Al Lajjun, Al Mafrag, Al Muqabalayn, Al Muwaqqar, Amman, As Salt, Ash Shawbak, Az Zarqa', Dayr Alla, Dead sea, Dhulayl, Dibbin, Ghawr Kabid, Irbid, Jarash, Juffayn, Khaww, King Talal Dam, Kufrayn, Mahis, Na'ur, Nahlah, Sadd al Khaldeyyah, Sahab, Shafa Badran, South Al Ghawr-Dead Sea, Ukaydir, Wadi al Arab, Wadi as Sir, Zayy, near the Zeizia.

*Collecting months.* JAN (7), FEB (4), MAR (30),

APR (69), MAY (28), JUN (13), JUL (7), AUG (7), SEP (0), OCT (12), NOV (19), DEC (2).

**Remarks.** The Painted Lady is a migrant butterfly distributed world-wide except most of South America. As the data indicate, it occurs in all parts of Jordan all months of the year. Larsen (1976) discussed its migration in the Middle East and emphasized the need for a more comprehensive data on its behavior. We observed large numbers migrating in north or north-western direction in February 1997 in Wadi Arabah and in the Jordan Valley. However, later in the season they were seen migrating in south or south-eastern direction. The butterflies fly very fast in open areas and very difficult to catch. But once they land on weeds in numbers they are easy to collect. They were so abundant that the wind shield of cars and radiators has to be washed after a short trip to the Jordan Valley. Amr et al. (1997) found this species to be common in Al Azraq Reserve. The normal food plants are species of *Carduus*, *Cynara*, *Arctium* and other Composites. Fabaceae and Brassicaceae are only used in crisis situations.

#### ***Polygonia egea* Cramer, 1775**

**Material.** 13 specimens. **Localities.** Al Jubayhah, Al Fuhays, Amman, As Salt, Ghawr Kabid.

**Collecting months.** MAY (4), JUN (5), JUL (0), AUG (1), SEP (1), OCT (1), NOV (1).

**Remarks.** The Southern Comma is found along the Mediterranean coast from Provence to Greece, through Turkey and the Levant to Afghanistan. In Jordan, this butterfly occurs mainly in the northern Mediterranean zone but may be found also in the Jordan Valley (at Gawr Kabid). Ten Hagen (1995) recorded it as far south as Petra. It has two or three broods from March to November. The food plants are species *Parietaria*.

#### ***Melitaea phoebe telona* Fruhstorfer, 1908**

**Material.** 61 specimens. **Localities.** Ajlun, Al Aridah, Al Bunayyat, Al Fuhays, Al Jubayhah, Al Mujib Nature Reserve, Al Quwasymah, Amman, As Salt, Athneebah, Ayn Abdah, Ayn Aqrabah, Ayn Qantarah, Az Zarqa, Dayr Alla, Jarash, Khaldia, Suwaylih, Tila' al Ali, Ukaydir, Umm al Hyran, Umm ar Rumman, Wadi al Arab, Wadi Jarash, Zayy, Ziqlab.

**Collecting months.** FEB (1), MAR (3), APR (35), MAY (10), JUN (10), AUG (1), SEP (1).

**Remarks.** The Knapweed Fritillary occurs from

North Africa and Spain to Korea. It was thought to inhabit the Mediterranean zones only, however, Fabiano (1998) recorded specimens from the arid granite mountains (southern desert) overlooking the town of Aqaba. The first brood flies in April, a second brood may occur late in the year but apparently in low numbers. Larsen (1974) found it on *Centaurea calcitrapa*, *Carduus pycnocephalus* in Lebanon.

#### ***Melitaea arduinna evanescens* Staudinger, 1886**

**Material.** 11 specimens. **Localities.** Al Quwasymah, Jarash, Shafa Badran, Tabarbawr, Umm ar Rumman.

**Collecting months.** MAR (5), APR (5), MAY (1).

**Remarks.** The Freyer's Fritillary is distributed from Bulgaria and Asia Minor to Iran and Central Asia. It was assumed that the subspecies *evanescens* is limited to As Salt area (Larsen & Nakamura 1983), however, we collected specimens from other areas like Jarash and Amman. Even though it was considered as a rare species, its numbers appear to be more than previously thought. A large number was observed flying in March at a sunny day in Tabarbawr (Amman). Ten Hagen (1995) recorded this species from Na'ur. The closest populations of this butterfly are in Iraq and southern Turkey. The Jordanian populations may be a relict of a brief period in time when there was a wet Irano-Turanian bridge between Jordan and Iraq (Larsen & Nakamura 1983).

#### ***Melitaea trivia syriaca* Rebel, 1905**

**Material.** 77 specimens. **Localities.** Ajlun, Ayn Qantara, Ayn Abdah, Ayn Aqraba, Bayt Yafa, Qu'aylibah, Ukaydir Wadi al Arab, Wadi Jarash, Ziqlab.

**Collecting months.** MAR (4), APR (43), MAY (15), JUN (13), JUL (0), AUG (0), SEP (0), OCT (2).

**Remarks.** The Mullein Fritillary occurs in hot parts of southern Europe through the Middle East to Baluchistan. In Jordan, it is common in the Mediterranean zones, Jordan Valley and fringes on eastern desert. Fabiano (1998) recorded specimens from the southern desert for the first time. Almost all of our records are in the northern Mediterranean zone. The data suggest a peak activity from April to June. The Larvae feed on *Verbascum* sp.

#### ***Melitaea deserticola macromaculata* Belter, 1934**

**Material.** 17 specimens. **Localities.** Al Jubayhah,

Al Mujib Nature Reserve, Ayn Qantara, Dayr Abu sa'id, Dayr Alla, Ghawr Kated, Juffayn, Tabaqat Fahl, Tila' al Ali, Wadi al Arab, Wadi Shua'yb, Zabdah.

*Collecting months.* MAR (3), APR (12), MAY (1), JUN (1).

*Remarks.* The Desert Fritillary occurs in North Africa and the Levant. It is found in the Mediterranean zones of Jordan, the fringes of the Jordan Valley and southern desert into Saudi Arabia (Pittaway 1985). Its flight is much higher above the ground than that of other Jordanian *Melitaea*. Larvae feed on species of Scrophulariaceae. Three broods are probable, the second and the third are partial and irregular (Larsen & Nakamura 1983).

### *Satyrinae*

#### *Melanargia titea titania* Calberla, 1891

*Material.* 387 specimens. *Localities.* Abu Nusayr, Afra, Ajlun, Al Jubayhah, Al-Karak, Al Mujib Nature Reserve, Al Muqabalayn, Al-Yazidiyah, Al Yadudah, Amman, Ar Rabad Castle, Ar Rajif, Ar Rumaymin, As Salt, As Salt, Az Zarqa, At Tafilah, Ayn Qantara, Amman, Birayn, Dana Reserve, Dibbin, Ghawr Kabid, Ibbin, Irbid, Jarash, Mahis, Mursi', Na'ur, Shafa Badran, Qwaylibah, Sakib, Ukaydir, Ar Rumman, Wadi al Arab, Wadi as Sir, Wadi Shu'ayb, Yajuz, Zeizia.

*Collecting months.* FEB (1), MAR (6), APR (76), MAY (268), JUN (15), JUL (0), AUG (17), SEP (0), OCT (3), NOV (1).

*Remarks.* The Palestine Marbled White occurs in the Levant. Larsen & Nakamura (1983) mentioned that it is limited in Jordan to the northern Mediterranean zone, but the record from At Tafila proves its occurrence in the southern Mediterranean zone also. Its peak activity appears to be in May as the above data suggest. A second brood is possible towards the end of the year. Larvae feed on grasses and adults are attracted to the flowers of *Carduus* and *Centaurea*.

#### *Hipparchia fatua sichaea* Lederer, 1857

*Material.* 6 specimens. *Localities.* Al Jubayhah, Ayn at Tannour, Irbid.

*Collecting months.* JUN (1), JUL (0), AUG (0), SEP (1), OCT (0), NOV (4).

*Remarks.* The Freyer's Grayling is a Ponto-mediterranean species, distributed from the Balkans

via the Middle East and Iran to Turkmenistan. A single brood occurs in June and July, while specimens collected later in the year are aestivating females who appear to oviposit at the onset of autumn. The food plants are grasses (Larsen & Nakamura 1983).

#### *Pseudotargumia pisidice* Klug, 1832

*Material.* 3 specimens. *Locality.* Al Mujib Nature Reserve, Wadi Shgeig.

*Collecting month.* JUL (3).

*Remarks.* The Sinai Grayling occurs in southern parts of Turkey, the Levant, Sinai, and as far south as Saudi Arabia (Pittaway, 1985). It was previously recorded from several localities in the northern Mediterranean zone only. The above localities represent the southernmost records in Jordan so far. It is possible that the species occurs in the southern Mediterranean zone as well. Larvae feed on grasses.

#### *Pseudochazara telephassa* Hubner, 1806

*Material.* 67 specimens. *Localities.* Al Jubayhah, Al Baq'ah, Al Karak, Al Mujib Nature Reserve, Amman, As Salt, Az Zarqa, Jarash, Shafa Badran, Suwaylih, Ar Rumman,

*Collecting months.* APR (1), MAY (25), JUN (25), JUL (15), AUG (1).

*Remarks.* The Telephassa Grayling is found in the Levant, Turkey, Iran and Afghanistan. It is the most common satyrid in Jordan occurring in both Mediterranean zones and eastern desert. Even though it was collected from June to August, Larsen & Nakamura (1983) mentioned records from October and they assumed a single protracted brood.

#### *Maniola telmessia* Zeller, 1847

*Material.* 62 specimens. *Localities.* Al Baq'ah, Al Jubayhah, Al Manshiyah Ar Rumaymin, As Salt, Ayn Qantara, Dayr Alla, Irbid, Jarash, Juffayn, Nahlah, Near Jarash, Suwaylih, Wadi al Arab, Wadi as Sir.

*Collecting months.* MAR (2), APR (20), MAY (26), JUN (3), JUL (1), AUG (9), SEP (1).

*Remarks.* The Eastern Meadow Brown is found in Turkey, Iran and the Levant. It is restricted to the northern Mediterranean zone. It has one brood in April and May. Specimens collected later in the year are aestivating individuals appearing to oviposit (Larsen & Nakamura 1983).

***Hyponephele lupinus centralis* Riley, 1921**

*Material.* 36 specimens. *Localities.* Al Jubayhah, Amman, As Salt, Jarash, Zayy.

*Collecting months.* MAY (4), JUN (26), JUL (5), AUG (1).

*Remarks.* The Oriental Meadow Brown occurs in North Africa, southern Europe, Asia Minor, the Levant, Iran, and Afghanistan. In Jordan, it appears to be limited to the northern Mediterranean zone. It has a single brood in May and June or July. Specimens collected in August or September are assumed to be aestivating individuals appearing to oviposit (Larsen & Nakamura, 1983). Larvae feed on grasses.

***Ypthima asterope* Klug, 1832**

*Material.* 52 specimens. *Localities.* Al Jubayhah, Al Mujib Nature Reserve, Ar Rumaymin, As Salt, Dayr Alla, Ghawr as Safi, Irbid, Kufrinijah, Madaba, Mahis, Ma'in, Marka, Nahlah, Tabaqat Fahl, Umm as Summaq, Wadi as Sir, Wadi Fayfa, Wadi Shu'ayb, Yajuz, Zayy.

*Collecting months.* MAR (3), APR (3), MAY (0), JUN (3), JUL (5), AUG (10), SEP (4), OCT (19), NOV (5).

*Remarks.* The African Ringlet is distributed in dry parts of tropical Africa, Arabia, and much of tropical Asia. It is common in the Mediterranean zones and the Jordan Valley. It appears to have many broods from March to November. Only one specimen is known from Al Azraq Reserve in the eastern desert (Amr et al. 1997).

***Lasiommata maera orientalis* Heyne, 1894**

*Material.* 12 specimens. *Localities.* Ar Rumaymin, As Salt, Jarash, Salalem As Salt, Tabarbawr.

*Collecting months.* APR (3), MAY (1), JUN (3), JUL (5).

*Remarks.* The Large Wall Brown occurs in North Africa, most of Europe, the Levant, the Middle east to the Himalayas. It was collected mainly in the northern Mediterranean zone from April to July, which may represent two broods, but Larsen & Nakamura (1983) expected a third late brood in September.

***Lasiommata megera emilyssa* Verity, 1919**

*Material.* 7 specimens. *Localities.* Ash Shawbak, Ayn Qantara, Ibbin, Kufrinijah, Sakib, Wadi al Haydan.

*Collecting months.* MAY (6), JUN (0), JUL (1).

*Remarks.* The Wall Brown is a Holo-mediterranean species. It was collected in the northern Mediterranean zone of Jordan, but also from Petra in the southern Mediterranean zone. It flies from February to August and probably to October (Larsen & Nakamura 1983).

**Lycaenidae*****Theclinae******Deudorix livia* Klug, 1834**

*Material.* 38 specimens. *Localities.* Al Jubayhah, Al Khirbah as Samra, Amman, Ar Rumaymin, Ar Rumman, As Salt, At Turrhah, Dayr Alla, Irbid, Nahlah, Near Jarash, Wadi Shu'ayb, Zayy.

*Collecting months.* JAN (1), FEB (0), MAR (0), APR (0), MAY (0), JUN (1), JUL (1), AUG (14), SEP (11), OCT (4), NOV (6).

*Remarks.* The Pomegranate Hairstreak (or Pomegranate Playboy) is distributed in the arid regions of Africa, the Arabian Peninsula, parts of the Middle East, and southwestern Iran. Previously collected from localities extending from Debbin in northern Jordan, as far as Aqaba in the south. It was collected from forested areas as well as from several localities in the Jordan Valley. It is quite common during August and September and declines towards December and then emerges again in early June. Larsen & Nakamura (1983) suggested a migratory status for this species, and indicated that autumn populations can persist. This is in agreement with the collecting dates of specimens taken from the Jordan Valley. *Deudorix livia* feeds on *Acacia farnesiana* and *Punica granatum*, where the former species is an introduced ornamental plant, commonly planted on roadsides in the Jordan Valley. Also, it feeds occasionally on olive flowers.

***Iolais glaucus* Butler, 1885**

*Material.* 1 specimen. *Locality.* Wadi As Salt.

*Collecting month.* OCT (1).

*Remarks.* The Arabian Sapphire is distributed throughout the Horn of African (Somalia and Ethiopia) and some parts of the Arabian Peninsula (Larsen 1983). It is associated with the striking flowers of *Loranthus* sp., a parasitic plant on *Acacia* trees.



*Aphnaeinae****Apharitis acamas acamas* Klug, 1834**

*Material.* 6 specimens. *Localities.* Al Jubayhah, Amman, Nahlah.

*Collecting months.* JUN (4), JUL (1), AUG (0), SEP (0), OCT (1).

*Remarks.* The Leopard Butterfly is an eremic species and several subspecies are recognized across the Sahara to India. Similar to the previous findings of Larsen & Nakamura (1983), it seems that the Leopard Butterfly occurs in northern Jordan from June to October while in the Jordan Valley, it can be found in December.

*Lycaeninae****Lycaena phlaeas timeus* Cramer, 1777**

*Material.* 46 specimens. *Localities.* Al Jubayhah, Amman, Ar Rumaymin, Al Aridah Road, As Simakiyah, Dayr Alla, Jarash, King Talal Dam, Kurayyimah, Nahlah, Tabaqat Fahl, Zabdah, Zayy.

*Collecting months.* FEB (1), MAR (6), APR (5), MAY (14), JUN (10), JUL (4), AUG (2), SEP (2), OCT (0), NOV (2).

*Remarks.* The Small Copper is found in the temperate Palaearctic region, Greenland and eastern North America. The subspecies *timeus* was collected from the Jordan Valley as well as from densely forested areas (Nahlah & Zayy). Apparently, it is a resident species and occurs throughout the months of the year, with high abundance in May and June. Larvae feed on the flowers of *Rumex* and *Polygonum*.

***Lycaena thersamon omphale* Klug, 1834**

*Material.* 261 specimens. *Localities.* Ajlun, Al Fuhays, Al Ghawr, Al Jubayhah, Al Khirbah as Samra, Amman, Ar Rumaymin, As Salt, Dayr Alla, Dhulayl, Dibbin, Jarash, Madaba, Nahlah, Suwaylih, Tila' al Ali, Umm ar Rumman, Umm as Summaq, Wadi Saqrah, Zayy.

*Collecting months.* MAR (2), APR (18), MAY (14), JUN (59), JUL (8), AUG (24), SEP (70), OCT (53), NOV (13).

*Remarks.* The Lesser Copper occurs from Italy and Austria to the Balkans, the Middle East and Afghanistan. Larsen & Nakamura (1983) discussed the subspecific forms of this species; *kurdistanica* and *omphale*, and concluded that the later is a valid subspecies for the Levant. It was collected from the

Mediterranean zones as well as from several localities within the Irano-Turanian zone. Collecting dates suggest two broods, one in April, followed by another in August. Adults prefer the flowers of *Eryngium creticum*, while larvae feed on *Rumex*, *Sarothamunus* and *Polygonum*.

*Polyommatainae****Lampides boeticus* Linnaeus, 1767**

*Material.* 55 specimens. *Localities.* Al Jubayhah, As Salt, Az Zarqa, Dead sea, Irbid, Jarash old road, Al Kirbah as Samrah, Zayy.

*Collecting months.* MAR (1), APR (4), MAY (2), JUN (6), JUL (9), AUG (7), SEP (14), OCT (12).

*Remarks.* The Long-tailed Blue is widely distributed in the Palearctic region from which it migrates into the Palaearctic region. It is found virtually in all types of habitats in Jordan. Collecting dates suggests that two broods emerge annually, one in May and June and another in September and October. Larsen (1974) stated that *L. boeticus* feeds on a wide range of legume species.

***Leptotes pirithous* Linnaeus, 1767**

*Material.* 61 specimens. *Localities.* Al Jubayhah, Az Zarqa', Ghawr Kabid.

*Collecting months.* JAN (5), FEB (0), MAR (0), APR (0), MAY (0), JUN (0), JUL (0), AUG (2), SEP (1), OCT (7), NOV (34), DEC (12).

*Remarks.* The Common Zebra Blue (or Lang's Short-tailed Blue) is an Afrotropical species that has succeeded in penetrating the Arabian Peninsula and southern Europe. It is common during early autumn to December in the Jordan Valley and disappears thereafter. Except for Al Jubayhah locality, the others are warm habitats. It was seen in large numbers in alfalfa fields in Gawr Kabid in the Jordan Valley.

***Tarucus rosaceus* Austaut, 1885**

*Material.* 5 specimens. *Localities.* Al Jubayhah, Iraq al Amir, Wadi as Sir, Wadi Shu'ayb.

*Collecting months.* MAR (1), APR (0), MAY (1), JUN (0), JUL (0), AUG (0), SEP (0), OCT (2), NOV (1).

*Remarks.* The Mediterranean Pierrot (or Mediterranean Tiger Blue) has a wide distribution, extending from North Africa to northwestern India. Localities indicated here are wadis with permanent water bodies that host a wide variety of wild flowers



all year round. The main food plant is *Ziziphus spina-christi*.

***Zizeeria karsandra karsandra* Moore, 1865**

*Material.* 11 specimens. *Localities.* Al Jubayhah, Ghawr Kabid.

*Collecting months.* JUL (1), AUG (0), SEP (1), OCT (4), NOV (5).

*Remarks.* The Asian Grass Blue is found from Australasia, via India, to Oman, Iraq, Lebanon, Egypt, Libya and Tunisia. It is common in the Jordan Valley, however, it was found to be local within the Mediterranean zones (Larsen & Nakamura 1983). It feeds on several Fabaceae species.

***Azanus jesous* Guérin-Ménéville, 1849**

*Material.* 1 example. *Localities.* Dayr Alla, Khunayzir dam.

*Collecting month.* MAY (1).

*Remarks.* African Babul Blue is a migrant butterfly found in Africa, Arabia, Middle East and India. It is rather common in warm habitats with water courses. It was collected previously from several localities along the Jordan Valley as well as from Aqaba. But it is not expected to be a permanent resident in Jordan (Larsen & Nakamura 1983). The food plant is *Acacia* spp. but *Prosopis* is a possible host.

***Chilades galba* Lederer, 1855**

*Material.* 4 specimens. *Localities.* Al Baqurah, Dayr Alla, Jawa.

*Collecting months.* MAY (3), JUN (1).

*Remarks.* The Lederer's Cupid is an eremic species with a wide range of distribution. It is a migrant species common in the Jordan Valley and was found locally in the northern Mediterranean zone and eastern desert. The food plants are *Prosopis* and *Acacia*.

***Chilades trochylus* Freyer, 1845**

*Material.* 24 specimens. *Localities.* Al Jubayhah, Amman, Irbid.

*Collecting months.* JUN (4), JUL (0), AUG (8), SEP (4), OCT (8).

*Remarks.* The Grass Jewel is found in Africa, the Middle East, the Balkans, Arabia, Iran, Afghanistan and north-western India. It was collected from several localities within all biogeographical regions of Jordan. Several broods are possible from April through October. Food consists of *Heliotropium* and

*Indigofera*.

***Plebejus pylaon cleopatra* Hemming, 1934**

*Material.* 56 specimens. *Localities.* Al Baqurah, Al Jubayhah, Al Khirbah as Samra, Amman, Ar Rumaymin, Ar Rumman, As Salt, Jarash, Jordan Valley, Mahis, Umm as Summaq.

*Collecting months.* MAR (1), APR (3), MAY (5), JUN (11), JUL (5), AUG (8), SEP (8), OCT (12), NOV (3).

*Remarks.* Three subspecies of the Zephyr Blue are known to occur in the Middle East. *Plebejus pylaon nichollae* Elwes 1901 in Lebanon, *Plebejus pylaon cleopatra* Hemming 1934 in southern Palestine, and *Plebejus pylaon philbyi* Graves 1925 originally described from Petra (Graves 1925; Hemming 1932; Larsen & Nakamura 1983). Larsen & Nakamura (1983) stated that two subspecies occur in Jordan; *P. p. cleopatra*, common in the transitional zone between the Mediterranean and the Irano-Turanian zones, and *P. p. philbyi* occurring in desert and arid habitats. Evidently, numbers of broods vary according to the biogeographical region; where as one brood appears in the spring in the Mediterranean zone, while two broods are laid in more warm and dry habitats. It feeds on *Astragalus* spp. (Fabaceae).

***Aricia agestis agestis* Denis & Schiffermuller, 1775**

*Material.* 13 specimens. *Localities.* Irbid, Qwaylibah.

*Collecting months.* JUN (1), JUL (0), AUG (2), SEP (9), OCT (1).

*Remarks.* The Brown Argus is found in Europe, the Levant and Iran. It appears to be a rare species in Jordan. It was collected previously from several localities within the northern Mediterranean zone of Jordan. The collecting dates suggest two broods, one in the spring and the second towards the end of the summer. It feeds on *Erodium* and *Helianthemum*.

***Polyommatus icarus zelleri* Verity, 1919**

*Material.* 150 specimens. *Localities.* Ajlun, Al Jubayhah, Al Khirbah as Samrah, Amman, Ar Rumaymin, Ar Rumman, As Salt, Dayr Alla, Irbid, Jarash Mahis, Na'ur, Near Aridah, Near Jarash, Ar Ramtha, Tila' al Ali, Umm as Summaq, Wadi Haydan, Yajuz, Zayy.

*Collecting months.* FEB (1), MAR (2), APR (20), MAY (16), JUN (22), JUL (17), AUG (27), SEP (21),

OCT (19), NOV (4), DEC (1).

*Remarks.* The Common Blue is common in North Africa, Europe, the Middle East and most of temperate Asia. It is the most common lycaenid in Jordan, inhabiting a wide range of habitats. It was collected from the northern and southern Mediterranean zones, Jordan Valley as well as desert habitats. Multiple broods are evident as the collecting dates indicate. These broods vary in number depending on the biogeographical zone. It was found to feed on *Lotus* and *Medicago*.

## Hesperiidae

### Pyrginae

#### *Pyrgus melotis melotis* Duponchel, 1834

*Material.* 7 specimens. *Localities.* Ar Rumaymin, Jarash, Jordan Valley, Nahlah.

*Collecting months.* APR (4), MAY (2), JUN (0), JUL (1).

*Remarks.* The Levantine Grizzled Skipper is found in the Levant and south-eastern Turkey. In Jordan, it is restricted to the northern Mediterranean zone, where it was previously collected from Debbin and Hammie. It prefers moist habitats such as small permanent springs bordered by *Rubus* (Larsen & Nakamura 1983). Apparently, one brood is deposited in the spring, while in Lebanon, Larsen (1974) indicated that two generations appear.

#### *Spialia orbifer hilaris* Staudinger, 1901

*Material.* (9) specimens. *Localities.* Al Jubayhah, Al Mujib Nature Reserve, Amman, Ar Rumaymin, Jarash, Madaba.

*Collecting months.* MAR (1), APR (2), MAY (3), JUN (1), JUL (1), AUG (0), SEP (1).

*Remarks.* The Orbiferous Skipper occurs in a series of subspecies in Yugoslavia, the Middle East, Russia, western China and Korea. In Jordan, it is confined to the northern Mediterranean zone. Larsen & Nakamura (1983) stated that two broods are produced, one in early April and the second in July. The food plant in Jordan is not known.

#### *Syrichthus tessellum nomas* Lederer, 1855

*Material.* 1 example. *Locality.* Jarash.

*Collecting month.* APR (1).

*Remarks.* The Tessellated Skipper can be found

from the Balkans via the Middle East to Central Asia. The subspecies *nomus* is rare in Jordan. So far, with this record, only 11 specimens were ever collected from Jordan, however, it is quite common in Palestine and Lebanon (Larsen & Nakamura 1983). Collected previously from Jarash, Ajlun and Wadi Zarka, from various types of habitats including olive groves. Most probably larvae feed on *Phlomis* spp.

#### *Syrichthus proto hieromax* Hemming, 1932

*Material.* 2 specimens. *Localities.* Al Jubayhah, Saad al kaldeyyah.

*Collecting month.* OCT (2).

*Remarks.* The Large Grizzled Skipper is a Mediterranean butterfly found in North Africa, Iberian Peninsula, Turkey and the Levant. The subspecies *hieromax* was originally described from Ajlun, Jordan (Hemming 1932), and seems to be localized in Jordan, Palestine and the coastal region of Lebanon. Larsen & Nakamura (1983) discussed the status of the two subspecies; *Syrichthus proto hieromax* is found in the coastal areas of Lebanon and it is rare in both Jordan and Palestine, and *Syrichthus proto lycaonius* is distributed in the Lebanese mountains. Larsen & Nakamura (1983) expected *Phlomis* spp. as hosts.

#### *Carcharodus alceae* Esper, 1780

*Material.* 72 specimens. *Localities.* Al Aridah, Al Jubayhah, Al Mushaqqar, Ar Rumaymin, As Salt, Dayr Alla, Ghawr as Aafi, Irbid, Jarash, Near Ghawr Kabid, Wadi Shu'ayb, Zayy.

*Collecting months.* FEB (1), MAR (2), APR (5), MAY (2), JUN (4), JUL (5), AUG (10), SEP (21), OCT (17), NOV (5).

*Remarks.* The Hollyhock Skipper (or the Mallow Skipper) is found in most of Europe, the Middle East, Afghanistan and north-western India. It is a common species in Jordan found almost all year round. Localities reported here are within the northern Mediterranean zone and the Jordan Valley. Also, collecting dates suggests that two broods are produced annually, one in early spring followed by one at the end of summer. The food plants are *Malva* and *Althaea*.

#### *Carcharodus stauderi ambigua* Verity, 1925

*Material.* 7 specimens. *Localities.* Al Fuhays, Al Jubayhah, Ar Rumaymin, Jarash, Ar Rumman.

*Collecting months.* APR (5), MAY (2).

**Remarks.** The North African Skipper is found in a series of subspecies from Morocco to the Levant, Asia Minor and western Iran. It was collected from several localities within the northern Mediterranean zone of Jordan, fringes of the eastern desert and the Jordan Valley. Specimens were collected during April and May, similar to findings indicated by (Larsen & Nakamura 1983).

### *Hesperiinae*

#### ***Thymelicus lineola fornax* Hemming, 1934**

**Material.** 89 specimens. **Localities.** Al Aridah Road, Al Jubayhah, Al Mushaqqar, Ar Rumaymin, As Salt, Dayr abu Sa'id, Irbid, Jarash, Juffayn, Kafr Yuba, Na'ur, Wadi al Arab, Wadi al Yabis, Zabdah.

**Collecting months.** MAR (17), APR (27), MAY (41), JUN (2), JUL (0), AUG (2).

**Remarks.** The Lulworth Skipper occurs in North Africa, Europe, Middle East and Central Asia. In Jordan, it is a common species, inhabiting both the Jordan Valley and the northern and southern Mediterranean zones. Peak collecting was in May. Specimens were reported further south as far as Petra. The food plants are grasses, especially the genera *Triticum* and *Arrhenatherum*.

#### ***Gegenes gambica* Mabilie, 1878**

**Material.** 30 specimens. **Localities.** Al Jubayhah, Al Mujib Nature Reserve, Ar Rumaymin, Ghawr Kabid, Hammamat Ma'en-Madaba, Irbid, Mujib Nature Reserve, Umm Qays.

**Collecting months.** MAR (1), APR (2), MAY (5), JUN (0), JUL (0), AUG (1), SEP (20), OCT (1).

**Remarks.** The Pigmy Skipper is an eastern Mediterranean butterfly. It was collected from several localities ranging from the Jordan Valley to the east in Zarka (Larsen & Nakamura 1983). Collecting dates suggest that the Pigmy Skipper have two broods, one in the spring and the other towards the end of the summer, with peak activity in September. Larvae feed on grasses.

#### ***Pelopidas thrax thrax* Hübner, 1821**

**Material.** 4 specimens. **Localities.** Hammamat Ma'en-Madaba, Near the Dead Sea.

**Collecting months.** MAY (2), JUN (0), JUL (1), AUG (1).

**Remarks.** The Millet Skipper occurs in Arabia, Egypt and the Middle East. It was previously

collected from the Jordan Valley as well as from northern Mediterranean zone mostly in September and November. It appears that the Millet Skipper is not common in Jordan. Larsen (1974) reported on the migratory behavior of this species in Lebanon, and perhaps its scarce population in Jordan represents migrants. Larvae are minor pest of rice in Iraq (Al Hussein 1984).

### **DISCUSSION**

A total of 65 species of butterflies are recorded from the different ecological zones in Jordan. With the two additional records, the total number of Jordanian butterflies is now 91 species and subspecies (Table 1). Composition wise, the butterflies of Jordan belong to five families. Family Lycaenidae includes the highest number of species (26 species and subspecies), the Pieridae (24 species and subspecies), the Nymphalidae (22 species), the Hesperidae (14 species), while the Papilionidae includes the least number (5 species).

Larsen (1987) analyzed the origin of the butterfly fauna of Jordan and the neighboring countries, where as 62%, 18% and 20% are of Palaearctic, Eremic and Tropical origins, respectively. However, Benyamini (1988) considered *Madais fausta*, *Zizeeria karsandra* and *Limenitis reducta* as oriental elements. No endemic species or subspecies are found in Jordan, since all originally described ones from Jordan were found later in nearby areas.

Since Jordan is mostly considered an arid country, where the Eastern Desert comprises up to 70% of the total area, 20 species (about one fourth of the total butterflies species) are migrants (Larsen & Nakamura 1983). Some of these migrants include; *Danaus chrysippus*, *Catopsilia florella*, *Euchloe ausonia*, *Madais fausta*, and *Junonia orithya*. Further analysis is required based on the current status of the existing butterflies.

After examining more than 3350 specimens of butterflies, and despite the continuous collecting over the past 7 years, we report 65 species, which is far less than the records given by Larsen & Nakamura (1983). Indeed, for the past three decades, the natural environment of Jordan has gone through extensive changes in the form of irrigated agriculture and urbanization due to population increase, where both activities resulted

in loss of natural habitats. Also, along with the expanding agriculture, uncontrolled misuse of pesticides certainly affected the insect fauna of Jordan, including butterflies. Livestock grazing, the use of wood for fuel, the accidental or deliberate burning of forest had also negative impact on the flora. For example, comparing the vegetation in Ash Shoumari Reserve in the Eastern Desert, Dibbin National Park in the northern Mediterranean zone and the vegetation in many mine fields along the borders in the Jordan Valley to the areas around them, clearly shows that grazing alone makes a great reduction in vegetation.

However, the absence of the unrecorded species does not necessarily indicate their extension. Some species are rare and have been recorded previously once or twice from one or two locations. For example, *Melitaea perseia sargon* was recorded from one specimen from Wadi Rajil. *Zegris eupheme tigris* was recorded from one specimen collected in Wadi Rum. *Catopsilia florella*, a migrant species found in tropical Africa, was recorded only twice in 50 years. *Iolana alferii* was also reported from one specimen collected in Petra. *Chazara persephone*, a Levantine species, was recorded once from the fringes of the eastern desert of Jordan in 1927. Other species are extremely local in distribution and occupy unique habitats which make them difficult to collect. For example, *Tomares nesimachus*, a Levantine species, is associated with a special type of soil on which the food plant, *Astragalus macrocarpus*, grows (Larsen & Nakamura 1983). *Papilio saharae* is found in the remote southern desert and its hill-topping behavior makes it hard to capture. Fabiano (1998) found that rainfall scarcity and irregularity heavily affected the amount of flying species as well as the quantity of individuals. The number of species recorded from the southern desert varied according to the rainfall from 13 in 1992 to a minimum of 7 in 1993 and up to 30 in the relatively wet spring in 1994, to fall again to 13 in 1995 and 8 in 1996. This may be the case also in other parts of Jordan especially in the eastern desert and in the Irano-Turanian zones.

Eight species of butterflies are considered rare in Jordan: *Anthocharis gruneri*, *Charaxes jasius*, *Limenitis reducta*, *Melitaea arduinna*, *Melitaea perseia*, *Chazara persephone*, *Tomares nesimachus* and *Aricia agestis*. The rare butterflies indicate areas that need to be subject of habitat conservation. According to the IUCN Red Data Book (Collins et al. 1985)

*Papilio alexanor* and *Archon apollinus* are locally and globally threatened. Further evaluation of the status of local species is urgently needed. The protection of such species must be a priority for nature conservationists. The public attention should be attracted to the importance of butterflies as a national heritage that must be protected by all means. Collecting butterflies should be restricted to only very common or pest species. Future research may be directed toward the assessment of the current distribution of the local butterflies, their association with the local plants as well as to identify the major threats.

Larsen & Nakamura (1983) has already mentioned that the tropical oases of the southern part of the Dead Sea area should have high priority for conservation. The vegetation of these oases is almost wholly tropical with a total of more than 40 species not found elsewhere in Jordan. This area has important butterflies hosts like *Cassia obovata*, *Zizyphus spina-christi*, *Moringa aptera*, *Salvadora persica* (food plant of *Colotis phisadia*) and *Calotropis procera* (main food plant of *Danaus chrysippus*). Since the Mediterranean zone is the absolute southern distribution limit for many Mediterranean species, we agree with Larsen & Nakamura (1983) who endorsed a suggestion by early workers that the forest of Wadi Al Hisha in this zone should be made a conservation area. Most of *Allancastris deyrollei*, *Archon apollinus*, *Melitaea phoebe*, *Lasiommata megera*, *Carcharodus orientalis*, *Thymelicus flava* and *Thymelicus lineola* reach Petra but are localized to a few good collecting spots where perennial water is available.

The recent peace treaties in the area has encouraged the establishment of many tourism projects and the constructing of several roads like Al Ardani Road on the eastern bank of the River Jordan and the road at eastern side of the Dead Sea. This may have negative impact on ecology of butterflies in these areas. However, in the first area, the Higher Counsel of science and Technology sponsored a research program in order to study the biodiversity of the concerned area among several others in order to lessen the negative impact on the biodiversity of the area. Recently, the Royal Society for the Conservation of Nature (RSCN) has established the Mujib Nature Reserve on the eastern side of the Dead Sea which is expected to play a major role in the preservation of many butterfly species in the area. Also in the Southern Desert,

the RSCN has initiated several studies on the fauna of Wadi Rum area including insects, which will help in making a managing plan which will protect the already poor natural habitats of the Wadi, but harbors interesting butterflies like *Zigris eupheme* and *Papilio saharae*.

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Table 1. List of the Butterflies of Jordan. Species marked with (\*) are recorded in this study.

#### Family Papilionidae

##### Subfamily Papilioninae

1. *Papilio machaon syriacus*\* Verity, 1905
2. *Papilio saharae* Oberthür, 1879
3. *Papilio alexanor maccabaeus*\* Staudinger, 1891

##### Subfamily Zerynthiinae

4. *Allanastria deyrhollei eisneri*\* Bernardi, 1971

##### Subfamily Parnassiinae

5. *Archon apollinus*\* Herbst, 1798

#### Family Pieridae

##### Subfamily Pierinae

6. *Aporia crataegi augustior*\* Graves, 1925
7. *Pieris napi dubiosa* Röber, 1907
8. *Pieris brassicae*\* Linnaeus, 1758
9. *Pieris rapae leucosoma*\* Schawerda, 1905
10. *Pontia edusa*\* Fabricius, 1777
11. *Pontia glauconome glauconome*\* Klug, 1829
12. *Madais fausta fausta*\* Olivier, 1804
13. *Colotis phisadia phisadia*\* Godart, 1819
14. *Colotis danae eupompe*\* Klug 1829. **New record**
15. *Colotis chrysonome chrysonome* Klug, 1829
16. *Belenois aurota aurota*\* Fabricius, 1793
17. *Euchloe ausonia melisander*\* Fruhstorfer, 1908
18. *Euchloe crameri aegyptiaca* Verity, 1911
19. *Euchloe belemia*\* Esper, 1799
20. *Euchloe falloui* Allard, 1867
21. *Euchloe charltonia*\* Donzel, 1842
22. *Zegris eupheme uarda*\* Hemming, 1929
23. *Zegris eupheme tigris* Riley, 1921
24. *Zegris eupheme larseni* Pittaway, 1985
25. *Anthocharis gruneri gruneri*\* Herrich-Schäffer, 1851.

##### New record

26. *Anthocharis cardamines phoenissa* von Kalchberg, 1894

##### Subfamily Coliadinae

27. *Catopsilia florella* Fabricius, 1775
28. *Colias crocea crocea*\* Geoffroy, 1785
29. *Gonepteryx cleopatra tauranica*\* Staudinger, 1881

#### Family Nymphalidae

##### Subfamily Danainae

30. *Danaus chrysippus chrysippus*\* L. 1758

##### Subfamily Charaxinae

31. *Charaxes jasius jasius*\* Linnaeus, 1767

##### Subfamily Nymphalinae

32. *Junonia orithya hera*\* Lang, 1884
33. *Limenitis reducta schiffmuelleri*\* Higgins, 1933
34. *Vanessa atalanta*\* L. 1758
35. *Vanessa cardui cardui*\* L. 1758
36. *Polygonia egea*\* Cramer, 1775
37. *Melitaea phoebe telona*\* Fruhstorfer, 1908
38. *Melitaea arduinna evanescens*\* Staudinger, 1886
39. *Melitaea trivialis syriaca*\* Rebel, 1905
40. *Melitaea perseia sargon* Hemming, 1932
41. *Melitaea deserticola macromaculata*\* Belter, 1934

##### Subfamily Satyrinae

42. *Melanargia titea titania*\* Calberla, 1891
43. *Hipparchia fatua sichaea*\* Lederer, 1857
44. *Pseudotargumia pisdice*\* Klug 1832

45. *Chazara persephone transiens* Zerny, 1932
46. *Pseudochazara telephassa*\* Hubner, 1806
47. *Maniola telmessia*\* Zeller, 1847
48. *Hyponphele lupinus centralis*\* Riley, 1921
49. *Ypthima asterope*\* Klug, 1832
50. *Lasiommata maera orientalis* Heyne, 1894
51. *Lasiommata megera emilyssa*\* Varity, 1919

#### Family Lycaenidae

##### Subfamily Theclinae

52. *Deudorix livia*\* Klug, 1834
53. *Iolau glaucus*\* Butler, 1885
54. *Tomares nesimachus* Oberthür, 1893

##### Subfamily Aphnaeinae

55. *Apharitis acamas acamas*\* Klug, 1834
56. *Apharitis myrmecophila* Dumont, 1922

##### Subfamily Lycaeninae

57. *Lycaena phlaeas timeus*\* Cramer, 1777
58. *Lycaena thersamon omphale*\* Klug, 1834

##### Subfamily Polymmatinae

59. *Anthene amarah amarah* Guerin-Meneville, 1847
60. *Lampides boeticus*\* L. 1767
61. *Leptotes pirithous*\* L. 1767
62. *Tarucus balkanicus* Freyer, 1845
63. *Tarucus rosaceus*\* Austaut, 1885
64. *Zizeeria karsandra karsandra*\* Moore, 1865
65. *Azanius jesous*\* Guerin-Meneville, 1849
66. *Azanius ubaldus* Cramer, 1782
67. *Pseudophilotes vicrama astabene* Hemming, 1932
68. *Pseudophilotes abencerragus nabataeus* Graves, 1925
69. *Iolana alferii* Wiltshire, 1948
70. *Chilades galba*\* Lederer, 1855
71. *Chilades trochylus*\* Freyer, 1845
72. *Plebejus pylaon nichollae* Elwes, 1901
73. *Plebejus pylaon cleopatra*\* Hemming, 1934
74. *Plebejus pylaon philbyi* Graves, 1925
75. *Aricia agestis agestis*\* Denis & Schiffermüller, 1775
76. *Polyommatus icarus zelleri*\* Verity, 1919
77. *Polyommatus locwii uranicola* Walker, 1870

#### Family Hesperidae

##### Subfamily Pyrginae

78. *Pyrgus melotis*\* Duponchel, 1834
79. *Spialia orbifer hilaris*\* Staudinger, 1901
80. *Spialia doris doris* Walker, 1870
81. *Syrictus tessellum nomas*\* Lederer, 1855
82. *Syrictus proto hieromax* Hemming, 1932
83. *Carcharodus alceae*\* Esper, 1780
84. *Carcharodus stauderi ambigua*\* Verity, 1925
85. *Carcharodus orientalis maccabaeus* Hemming, 1925

##### Subfamily Hesperinae

86. *Thymelicus acteon phoenix* Graves, 1925
87. *Thymelicus flava syriaca* Tutt, 1905
88. *Thymelicus lineola fornax*\* Hemming, 1934
89. *Gegenes nostrodamus* F., 1793
90. *Gegenes gambica*\* Mabille, 1878
91. *Pelopidas thrax thrax*\* Hübner, 1821

# Shelter building in the Hesperiiidae: A classification scheme for larval shelters

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**Abstract:** The majority of larvae in the family Hesperfiidae build and inhabit shelters on or near the host plant. A review of hesperfid natural history publications from all regions, in combination with extensive field observations on New World skippers shows that larval shelters fall into at least ten separate categories. The functions, plasticity, and ontogenetic variation of skipper shelters are discussed. Terms useful in shelter description are defined and a classification system for shelter types is suggested. A dichotomous key to these types is provided.

**Key words:** life history, larva, skipper, shelter terminology, leaf roll

## INTRODUCTION

The exophytic larvae of numerous lepidopteran families construct and inhabit shelters made, at least in part, from host plant leaves. Caterpillars roll, fold, tie, or web leaves to make a diverse array of structural retreats (DeVries 1987, Scoble 1992, Stehr 1987). Many of these (ie. Thyrididae, Gelechiidae, Pyralidae, Tortricidae, Lasiocampidae, Oecophoridae, Nymphalidae, Hesperiiidae) can be separated into families by their shelters alone (L. A. Dyer pers. com.). The family Hesperiiidae comprises over 3,000 species of butterflies (Munroe 1982) and likely contains the greatest diversity of larval shelters within any lepidopteran family. The diversity of shelter types built, however, has remained largely ignored. While natural history studies on immature skipper stages devote much attention to physical characteristics of the larvae, they often fail to accurately describe larval shelters. Not surprisingly, there is no clear method or vocabulary for describing these shelters. Our current understanding of the pattern and process of shelter building by skipper larvae remains weak, despite detailed observations from

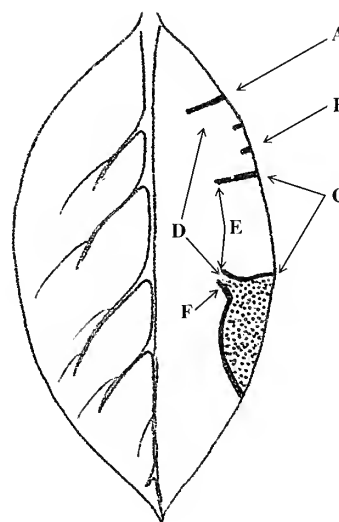


Fig. 1. Shelter parts and terminology: Examples of shelter cut patterns for Type 9 (top) and Type 10 (bottom) shelters; stippled area = shelter lid; a) Major cut b) Minor cut c) Proximal portion of major cut d) Shelter bridge e) Distal portion of major cut f) Shelter stem.



early natural historians (e.g. Scudder 1889; Moss 1949).

While a few skippers are reported to live and feed exposed during at least one larval instar (Moss 1949, Scudder 1889, J. Brock pers. comm.), the vast majority construct leaf shelters and spend the majority of their time inside them (eg. Atkins 1975, Atkins & Miller 1977; Moss 1949, Scudder 1889; Young 1991). Despite the time spent inside shelters, frass rarely accumulates within (Greeney & Jones pers. obs.). Using a sclerotized anal comb (Scoble 1992), most skippers forcefully eject frass away from the shelter (Greeney & Jones pers. obs.). A single exception from the literature is described by Scudder (1889) as 'soiling its nest considerably.' Despite the apparent uniformity in larval shelter use, however, shelter architecture can vary greatly across species and between larval instars.

The majority of species described in the literature or observed in the field make at least two separate shelter types during their larval life (eg. Atkins 1988, Williams & Atkins 1996, 1997; Atkins et al. 1991, Graham 1988; Miller 1990; Young 1993), but this ontogenetic change is rarely documented in detail.

Natural history characters can provide useful data for creating phylogenetic hypotheses (DeVries 1987; Hennig 1966). However, the lack of detailed information on shelters makes them unavailable for use in phylogenetic analyses. A review of skipper natural history literature in combination with field observations indicates that some shelters may be diagnostic at species or higher taxonomic levels (Greeney pers. obs.), suggesting they may be important, yet unexplored, phylogenetic characters. By synthesizing field observations and published literature, this study proposes a standardized terminology for describing shelters and presents a dichotomous key for classifying known shelter types.

## MATERIALS AND METHODS

During the past 10 years, observations on skipper natural history and shelter construction were conducted on over 200 species at the following locations: Yanayacu Biological Station and Center for Creative Studies, Cosanga, Napo, Ecuador (YBS); Sacha Lodge Research Station, Sucumbios, Ecuador (SLRS); La Selva Lodge Biological Station, Sucumbios, Ecuador (LS); El Monte Biological

Station, Pichincha, Ecuador (EM); Rio Palenque Biological Station, Santo Domingo, Ecuador (RPBS); Tinalandia Lodge, Santo Domingo, Ecuador (TL); Jatun Sacha Biological Station, Napo, Ecuador (JSBS); Celica, Loja, Ecuador (CE); Las Alturas Biological Station, San Vito, Costa Rica (LABS); La Selva Biological Station, Costa Rica (LSBS); Alamos, Sonora, Mexico (AM); Mt. Lemmon, Pima County, Arizona, USA (ML); Tucson, Pima County, Arizona, USA (TA); Oasis State Park, New Mexico, USA (OSP); Springfield, Hampshire County, West Virginia, USA (SWV); Georgetown University, Washington DC, USA (GU).

For all hesperiid larvae found in the field we carefully described the form and structure of each shelter and made detailed drawings in nature. Larvae were then brought to the lab for further observations or left *in situ* and observed over the lifetime of the larva. To avoid potential laboratory artifacts affecting shelter construction behavior, only those shelters made by larvae living in the field were considered as legitimate shelter types. Whenever possible, behavior associated with shelter construction was observed and recorded.

To further assess the diversity of shelters we thoroughly examined and compared descriptions and illustrations in the natural history literature. Particular attention was given to those publications with graphic illustrations of shelters (ie. Comstock & Comstock 1943; Fox et al. 1965, Holland 1898; Janzen & Hallwachs 2000; Johnston & Johnston 1980; Larsen 1991; Morris 1980; Riley 1975; Smith et al. 1994; Weed 1917), and these figures were compared with shelters studied in the field. We then used literature and field observations to build a dichotomous key to distinct shelter types. The Megathyminae, whose larvae tunnel inside plant tissues (Scoble 1992), were not included in this study.

## RESULTS

Skipper shelters range from a simple resting spot at the base of a leaf secured by a few strands of silk (Fig. 2f), to the elaborately peaked and perforated structures of others (eg. Figs. 2d, 3g, 3h, 4b, 4c). All known shelters, however, may be easily classified into 10 different types using the dichotomous key provided in Appendix A. These types then fall into



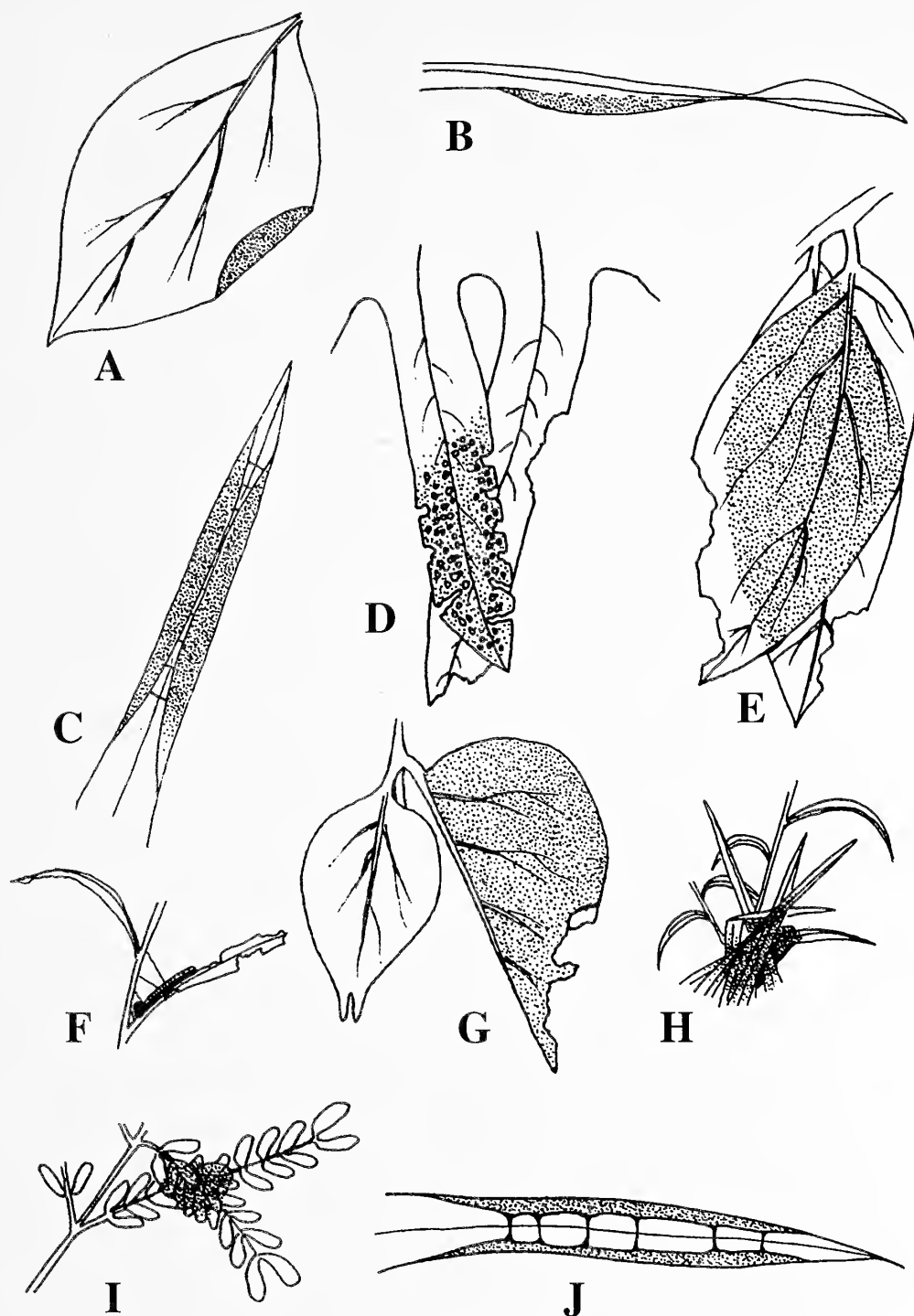


Fig. 2. Group I Shelters: Shelters not always drawn on actual host plant leaf and not drawn to scale; stippled areas indicate the portion of the leaf or leaves which have been manipulated to hide the larva; a) Type 2 folded to ABS, ABV, *Aroma aroma* Hew. (Pyrg), fifth instar, *Cyclanthus* Poit. (Cyclanthaceae), LS; b) Type 2 folded to ABS, LV, *Aroma aroma* Hew. (Pyrg), fifth instar, *Cyclanthus* (Cyclanthaceae), LS; c) Type 2, ADV, unknown Hesp, fifth instar, unknown Poaceae, LS; d) Type 4 with perforations and channels, ADV, *Eracon paulinus* Stoll (Pyrg), fifth instar, unknown hosplant, LS; e) Type 4, ADV, *Eantis thraso* Hübner (Pyrg), fifth instar, *Citrus* L., LS; f) Type 1, LV showing position of larva, *Atalopedes campestris* Boisduval, first instar, *Digitaria sanguinalis* L. (Poaceae), GU; g) Type 2, LV, unknown Pyrg, fifth instar, *Bauhinia* L., LS; h) Type 3, unknown Hesp, fifth instar, unknown Poaceae, LS; i) Type 3, ADV, unknown Pyrg, third instar, unknown Leguminoidea, LS; j) Type 2, ADV, *Atalopedes campestris* (Boisduval), second instar, *Digitaria sanguinalis* (Poaceae), GU; Abbreviations: : For locality abbreviations see Material and Methods, ABS=abaxial surface, ABV=abaxial view, ADV=adaxial view, LV=lateral view, Hesp=Hesperiinae, Pyrg=Pyrginae.

three major groups; no-cut, one-cut, and two-cut shelters.

**Group I Shelters (no-cut shelters, Types 1-4).**

Group I shelters are formed without any initial cuts made in the leaf. The simplest, Type 1 shelters (rudimentary shelter, Fig. 2f), consist simply of a loosely silked area where the larvae return between feeding bouts. They are defined as areas where resting occurs and silk is deposited, but where the position of the foodplant is not necessarily modified. Often Type 1 shelters consist of merely a small mat of silk about a body length long, and those species reported to lack shelters should be observed carefully to determine if they in fact create such mat of resting silk.

Type 2 shelters (no-cut fold, Figs. 2a, b, c, g, j) encompass a diversity of forms dictated in part by leaf shape, size, and thickness. They are formed using only one leaf or leaflet. Often they are more tubular in shape than most shelters (Figs. 2c, j), but can be flattened as well (Fig. 2g). They vary from a leaf edge slightly curled over or under the leaf blade (Figs. 2a, b) to shelters where opposite leaf margins are drawn together to form a tube. This latter type is common among grass feeding hesperiines. Another Type 2 shelter seen in many late instar pyrgines and also formed by drawing opposite leaf margins together, creates a shallow pocket when the leaf is folded along the midvein (Fig. 2g).

Type 3 shelters (multi-leaf shelters, Figs. 2h, i) are typically a disorganized cluster of many plant parts. All Type 3 shelters are composed of more than two leaves, leaflets, or leaf lobes, and are most commonly found on plant species where the size of the leaves or leaflets is too small to accommodate larger larvae. This is a common type, and is often constructed by late instar larvae feeding on grasses (Fig. 2h) or pinnate legumes (Fig. 2i).

Type 4 shelters (two-leaf shelter, Figs. 2d, e, 3g) are formed using only two leaves (Fig. 2e), leaflets (Fig. 3g), or leaf lobes (Fig. 2d). The two blades are most often slid over so that the pocket formed by the overlap is composed of an adaxial leaf surface opposing an abaxial surface. They may, however, be flipped such that one surface opposes the same surface on the other leaf.

**Group II Shelters (one-cut shelters, Types 5-7).**

Group II shelters are constructed using only one major cut. The most easily recognized of this shelter group are Type 5 (center-cut fold, Fig. 3b). In these

unique shelters, the major cut is initiated from the center of the leaf and does not begin at the leaf margin as in all other shelter types. Cuts range from circular to oval and the shelter lid may be flipped on to the adaxial or abaxial surface of the leaf.

Type 6 shelters (one-cut fold, Figs. 3a, d, e, f) are the most common type of Group II shelters. They vary from being tightly silked and flattened (Figs. 3a, d) to more loosely silked and conical or tubular in overall form (Figs. 3e, f). In the latter cases, the larvae are often visible from at least one angle. They may be formed on the adaxial (Figs. 3a, f) or abaxial (Figs. 3d, e) surface.

Type 7 shelters (one-cut slide, Fig. 3c) have been observed only once, and it is unknown how commonly they occur. They are formed by one major cut, which allows the portions of the leaf on opposite sides of the cut to be slid over top one another. In Type 7 shelters the abaxial surface of the leaf opposes the adaxial surface.

**Group III Shelters (two-cut shelters, Types 8-10).**

All Group III shelters are constructed using only two major cuts. The three types are separated by the relative position of these two cuts to one another. Type 8 shelters (two-cut fold, Figs. 4d, e, f) differ from other Group III shelters by having the major cuts beginning on opposite sides of the leaf midvein. They typically take on two forms, but both are created by drawing together opposite leaf margins. This results in a tube-shaped (Figs. 4d, e) or flattened pocket (Fig. 4f). In many grass feeding Hesperines these shelters are often further modified using a positioning cut along the midvein. Frequently the mid vein, at the site of the positioning cut, is subsequently silked so as to firmly hold the shelter out of the plane of the rest of the leaf blade. This often results in the leaf looking diseased or dead (Fig. 4e).

Type 9 shelters (two-cut unstemmed fold, Figs. 3i, 4g, i, j) are similar to Type 10 (two-cut stemmed fold, Figs. 3h, 4a, b, c, h) shelters but are separated by the relative positions of the distal portions of the respective major cuts. Type 9 shelters have the distal ends of the major cuts separated by more than one half the distance between the proximal ends and are folded along a broad shelter bridge. They are often square, or nearly so, in overall shape. In contrast, Type 10 shelters, the distal ends of the two major cuts are separated by no more than one half the distance that separates the proximal ends. This

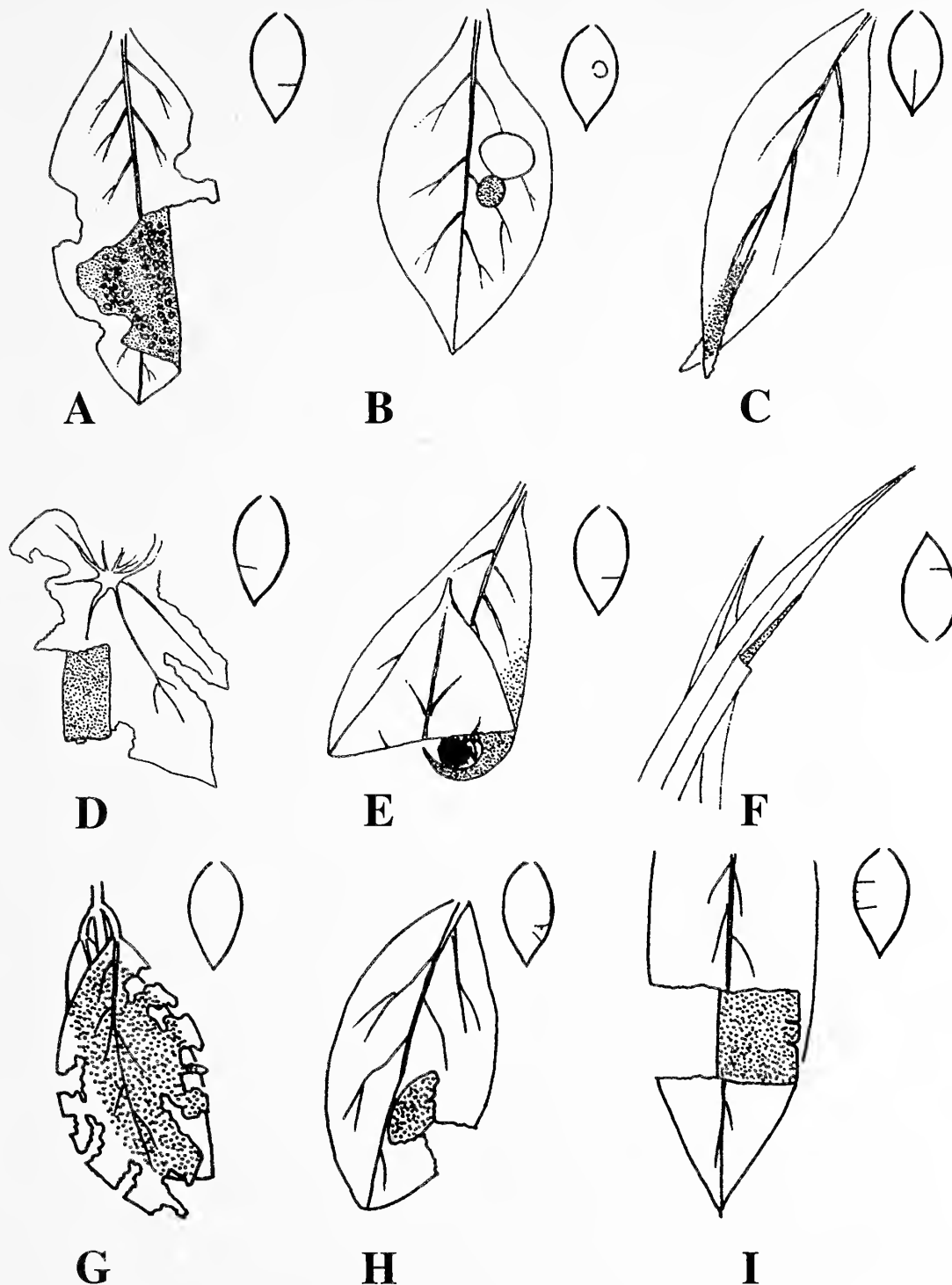


Fig. 3. Group II, Group I, and Group III Shelters: Stylized leaf-outline insets show the positions of major and minor cuts; shelters not always drawn on actual host plant leaf and not drawn to scale; stippled areas indicate the portion of the leaf or leaves which have been manipulated to hide the larva; a) flattened Type 5 (Group II) with perforations, ADV, *Quadrus cerialis* Stoll (Pyrg), fifth instar, *Piper* L. (Piperaceae), LS; b) Type 6 (Group II), ADV, unknown Phyrropyginae, first instar, *Vismia* Vand. (Guttiferaceae), YBS; c) Type 7 (Group II), ADV, unknown Pyrg, fifth instar, *Deffembachia* Schott (Araceae), LS; d) Type 5 (Group II) folded to ABS, ABV, *Systasea zampa* W. H. Edwards (Pyrg), fifth instar, *Abutilon sonora* Gray (Malvaceae), TA; e) Type 5 (Group II) folded to ABS, anterior view showing position of larva, unknown Pyrg, fifth instar, unknown foodplant, LS; f) Type 5 (Group II), ADV, *Turesis basta* Evans (Hesp), first instar, unknown Poaceae, LS; g) Type 4 (Group I) with channels, ADV, *Erynnis* Schrank, fifth instar, *Robinia* L. (Leguminoidea), SWV; h) Type 10 Group III with one-secondary-cut tent, ADV, *Epargyreus clarus* Cramer (Pyrg), second instar, *Robinia* L. (Leguminoidea), ML; i) Type 9 (Group III) with two-secondary-cut tent, ADV, *Entheus latebrosus* Austin, fourth instar, *Grias* L. (Lethycidaceae), LS. Abbreviations: For locality abbreviations see Material and Methods, ABS=abaxial surface, ABV=abaxial view, ADV=adaxial view, Hesp=Hesperiinae, Pyrg=Pyrginae.

results in a shelter that is folded over along a narrowed shelter bridge and often gives them a stemmed appearance. Often this shelter stem is nothing more than a single, secondary leaf vein.

**Terminology.** During attempts to adequately describe skipper shelters it became obvious that a unique set of terms was needed for standardization. Below we provide terms useful in describing and differentiating larval shelters.

**Cuts.** **MAJOR CUT** (Fig. 1a). Any cut essential to the formation of the basic shelter type and which is also more than twice the length of the larval head capsule width (at the time of shelter construction). **POSITIONING CUT.** Defined as a cut, usually away from the shelter itself, that enables alteration of the shelter's position in relation to the rest of the plant. Generally it functions like (or with) positioning silk. **PROXIMAL PORTION OF A CUT** (Fig. 1c). This is the section of the cut where cutting begins and, with the exception of Type 5 shelters (Fig. 3b), is the portion where the cut meets the leaf margin. **DISTAL PORTION OF A CUT** (Fig. 1e). This is the opposite end of the major cut from the proximal portion and, in all described shelters, is located away from the leaf margin. Unless shelter construction is observed, distal and proximal portions are generally not identifiable in Type 5 shelters or in positioning cuts.

**Shelter parts.** **SHELTER LID** (Fig. 1). This is the portion of the shelter (in Group II and III, except Type 8) which has been cut away and manipulated into position to oppose the rest of the leaf blade and hide the larva. Shelter lids may be on the abaxial or adaxial surface of the leaf. Group I and Type 8 shelters, as they are often too disorganized or rudimentary to describe in detail, do not have a shelter lid. **SHELTER BRIDGE** (Fig. 1d). The portion of the shelter (in Group II and III, except Type 7), near the distal portions of the major cuts, along which it is folded. **SHELTER STEM** (Fig. 1f). The part of Type 9 shelters, where the distal portions of the major cuts are parallel, or nearly so. This is often a single, secondary leaf vein and is the portion of the shelter between the bridge and the lid.

**Shelter modifications.** **PERFORATIONS.** Perforations are modifications made by feeding damage that do not alter the overall shape of the shelter (Fig. 3a). They differ from channels by never beginning from a leaf margin or cut edge, and are usually circular or oval in form. **CHANNELS.** Channels are similar to perforations, but begin from a leaf margin or cut

edge (Figs. 3g, 4b). Like perforations, they do not alter the overall form of the shelter. Shelters may be modified with both perforations and channels (Fig. 2d). **TENTED SHELTERS.** These are shelters which often, but not always, include minor cuts that allow the shelter to take on a domed or peaked shape. This is created by silking together the opposite sides of a minor cut, or sometimes a pinched together section of the leaf, which draws that portion of the shelter into a peak or crease. Tented shelters may be formed without, or with one (Fig. 3h) or multiple (Figs. 3i, 4c) minor cuts. **MULTIPLE LIDDED SHELTERS.** These shelters may be of any type in overall construction but, due to additional feeding damage near the shelter, are partially or completely obscured by one or several flaps of leaf (Fig. 4g). It is not known if this is intentional, but in those species observed to live in multiple lidded shelters this was seen in over 90% of the shelters created (Greeney, unpubl. data).

**Shelter silk.** The following terms refer to the location or function of silk laid down by the shelter builder and do not necessarily reflect any differences in chemical composition. Silk laying patterns alone can be useful in differentiating shelters built by different species (Greeney unpubl. data). **POSITIONING SILK.** Positioning silk is that used to alter the overall position of the shelter in relation to the host plant. It does not alter the basic form of the shelter, but simply moves the entire shelter out of its natural position. Positioning silk is commonly used in grass feeding hesperines (Fig. 4e), but has also been observed in at least one species of pyrgine (Greeney & Warren in prep.). It is frequently located at the site of a positioning cut. **SEALING SILK.** This refers to any silk laid in the process of creating the basic shelter shape, moving the shelter lid, or sealing various pieces together. In shelter Types 9 and 10, sealing silk is generally laid at or near the shelter bridge to begin flipping the shelter lid. As the lid approaches the leaf surface, often more sealing silk is then spun between the lid edge and the leaf to draw it into its final position (H. Greeney unpubl. data). In Type 3 shelters, sealing silk is used to draw and anchor the various parts of the leaf or plant together (Figs. 2h, i). Often, many strands of sealing silk form easily visible ties that hold the shelter together (Fig. 2j). **RESTING SILK.** This is defined as silk spun inside the shelter which does little or nothing to contribute to the shape or

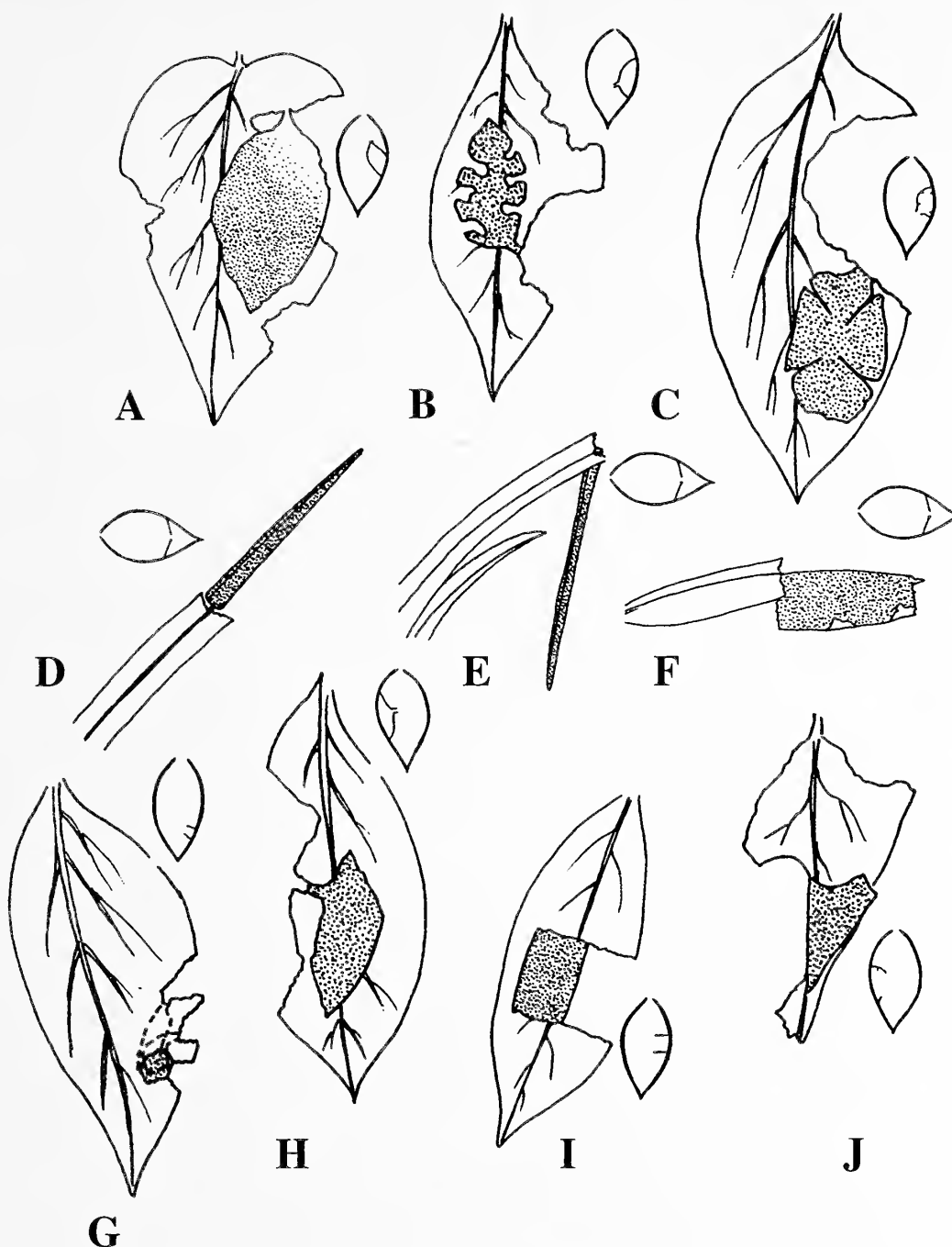


Fig. 4. Group III Shelters: Stylized leaf-outline insets show the positions of major and minor cuts; shelters not always drawn on actual host plant leaf and not drawn to scale; stippled areas indicate the portion of the leaf or leaves which have been manipulated to hide the larva; dashed lines indicate portions of the leaf not involved in shelter construction, but which obscure the shelter in some fashion; a) Type 10 slid rather than folded, ADV, unknown Pyrg, fifth instar, *Inga* Mill. (Mimosaceae), LS; b) Type 10 with channels, ADV, unknown Pyrg, fourth instar, *Inga* (Mimosaceae), LS; c) Type 10 with Four-minor-cut tent folded to ABS, perforations present but not illustrated for clarity of minor cuts, ABV, unknown Pyrg, fifth instar, unknown foodplant, LS; d) Type 8 tubular form, ADV, unknown Hesp, fourth instar, unknown Poaceae, LS; e) Type 8 tubular form in hanging position, LV, *Turesis* Godman (Hesp), fifth instar, unknown Poaceae, LS; f) Type 8 flattened form, LV, *Salliana* Evans (Hesp), fifth instar, *Calathea* G. Mey. (Marantaceae), LS; g) Type 9 with multiple lids, ADV, *Entheus latebrosus* Austin, first instar, *Grias* (Lethycidaceae), LS; h) Type 10, ADV, *Celaenorrhinus jao* Mabilie (Pyrg), fourth instar, unknown Acanthaceae, SLRS; i) Type 9 folded to ABS, ABV, *Astrartes talus* Cramer (Pyrg), fifth instar, unknown foodplant, LS; j) Type 9, ADV, *Hesperopsis* Edwards (Pyrg), third instar, *Atriplex* L. (Chenopodiaceae), TA. Abbreviations: For locality abbreviations see Material and Methods, ABS=abaxial surface, ABV=abaxial view, ADV=adaxial view, LV=lateral view, Hesp=Hesperiinae, Pyrg=Pyrginae.

position of the shelter. Resting silk is generally used by larvae for attaching their crochets while inside the shelter (Greeney & Jones pers. obs.).

## DISCUSSION

That larval shelters serve important functions is evidenced by the numbers and diversity of lepidopteran families known to create shelters (DeVries 1987, 1997; Scoble 1992; Steh 1987). Leaf shelters may reduce predation (Damman 1987; Eubanks et al. 1997; Jones 1999; Loeffler 1996), alter the interior microclimate by providing shade and/or increasing humidity (Henson 1958), or prevent desiccation (Jones pers. obs.). Rolling or tying leaves may also increase leaf nutritional quality (Sagers 1992), allow larvae to feed on phototoxic plants (Sandberg & Berenbaum 1989), and protect caterpillars from dislodgment (Loeffler 1996). Additional modifications to the basic structure, such as channels or perforations (Figs. 2d, 3a, 3g, 4b), may also serve various functions such as draining the shelter of rainwater or enhancing air circulation inside the shelter and preventing the buildup of pathogenetic bacteria and fungi (Scudder 1889; Young 1991).

In contrast to the uniformity that skipper species or larval instars show in nature, various authors have recorded larvae in the lab using portions of their rearing container in shelter construction (Jones 1999; Scudder 1889; Young 1993). In the field, larvae have been recorded to include adjacent leaves, other parts of non-foodplants, and even nearby detritus (Clark 1936; Jones 1999; Atkins 1987; Williams & Atkins 1997). Manipulative experiments will be of key importance in elucidating the cues used for choosing building materials and constructing shelters.

Despite the extensive number of papers reviewed and the copious field observations used to define these ten shelter types, the unexplored diversity of larval hesperiids and the lack of detailed descriptions in the literature all but guarantee the discovery of further shelter types or groups. The intent of this paper is not to show that all larval shelters will easily fall into one of these ten types, but rather to create a standardized method for describing shelters and discussing shelter construction. It is the hope of the authors that this manuscript will encourage

future studies to more thoroughly describe hesperiid shelters and allow for easier comparison and discussion of these ecologically interesting structures. Using this organization of shelter types, we encourage the careful examination and categorization of others in the hopes that shelter construction may prove useful in resolving hesperiid phylogenies.

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## APPENDIX A

### Dichotomous key to larval shelter types

- 1a. Shelter mostly or entirely hiding larvae from view (2)
  - 1b. Shelter rudimentary, barely concealing larvae, usually consists of a naturally formed pocket or crevice on plant or an area where resting silk has been laid that begins to curl leaf blade, shelter area mostly unmodified by larvae (**Type 1 shelter; rudimentary shelter**; Fig. 2f)
  - 2a. Shelter construction involving one or more cuts in the leaf (5)
  - 2b. Shelter construction not involving cutting of leaf (excluding feeding damage modifications such as perforations or channels eaten from shelter structure) (3)
  - 3a. More than one leaf, leaflet, leaf-lobe, or plant part involved in the shelter construction (4)
  - 3b. Only one leaf involved in shelter construction, typically a rolled leaf, one folded in half along the mid-vein, or simply the edge curled over or under the blade (**Type 2 shelter; no-cut fold**; Figs. 2a, b, c, g, j)
  - 4a. More than two leaves, leaflets, leaf-lobes, or plant parts involved, typically a disorganized appearing cluster of small leaves or leaflets silked together, common among grass feeding larvae (**Type 3 shelter; multi-leaf shelter**; Figs. 2h, i)
  - 4b. Only two leaves, leaflets, or leaf lobes involved in shelter design, many be slid over and silked ventral surface to dorsal surface or flipped over with dorsal surface to dorsal surface or ventral surface to ventral surface (**Type 4 shelter; two-leaf shelter**; Figs. 2d, e, 3g)
  - 5a. At least one cut begins from the leaf margin (6)
  - 5b. No cuts are initiated from leaf margin, shelter usually rounded and folded over along a narrow or stemmed section of leaf (**Type 5 shelter; center-cut fold**; Fig. 3b)
  - 6a. Shelter construction involving only one major cut (7)
  - 6b. Shelter involving more than one major cut (8)
  - 7a. Major cut begins at leaf margin, leaf flap curled or folded onto abaxial or adaxial surface of leaf, shelter formed so that abaxial leaf surface opposes abaxial surface or adaxial surface to adaxial surface (**Type 6 shelter; one-cut fold**; Fig. 3a, d, e, f)
  - 7b. Shelter as in 7a, but one section of leaf slid over or under another part, shelter formed so that an abaxial leaf surface opposes an adaxial surface (**Type 7 shelter; one-cut slide**; Fig. 3c)
  - 8a. Shelter with two major cuts, usually both beginning on same side of leaf mid-vein, if on opposite sides of mid-vein then shelter is at apex of leaf and has a definite top and bottom, shelter lid may be folded over along a narrow or broad leaf section (9)
  - 8b. Shelter not as above, two major cuts involved in construction, major cuts always beginning on opposite sides of the mid-vein and typically reach to mid-vein, shelter may be flattened (Fig. 4f) or tubular (Fig. 4d), may hang from end of leaf (Fig. 4e) (**Type 8 shelter; two-cut fold**; Figs. 4d, e, f)
  - 9a. Shelter folded or curled; portion of leaf along which shelter is folded or rolled is more than half the length of the leaf margin portion of the shelter lid; shelter usually square or rectangular; may be folded or curled onto abaxial or adaxial surface of the leaf (**Type 9 shelter; two-cut, unstemmed fold**; Figs. 3i, 4i, j)
  - 9b. Shelter folded, curled, or slid; portion of leaf along which shelter is hinged is less than half the width of the leaf margin portion of the shelter lid; often stemmed in appearance; shelter usually triangular, trapezoidal, or rounded; may be folded or slid onto abaxial or adaxial surface of leaf (**Type 10 shelter; two-cut, stemmed fold**; Figs. 3h, 4a, b, c, g, h)
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# Seasonal fluctuation and mortality schedule for immatures of *Hypna clytemnestra* (Butler), an uncommon neotropical butterfly (Nymphalidae: Charaxinae)

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**Abstract.** I describe the pattern of abundance and a mortality schedule for a population of *Hypna clytemnestra huebneri* (Butler) (Nymphalidae: Charaxinae), an uncommon neotropical butterfly inhabiting southeastern Brazil. Over a one year period I gathered data on the abundance of immatures through periodic censuses on 65 host plants, whereas adults were trapped monthly. A marked seasonal variation in abundance of immatures was detected, with a peak during the wet season. Adults were present throughout the year, but at very low numbers. The construction of a life-table showed that the main mortality factors acting upon immatures were parasitoids and predators. Eggs and medium to large-sized larvae suffered higher mortality, characterizing a mixed Type II/III survivorship curve. The data highlight the effect of predators upon larval stage as well as the great impact of parasitoids on eggs, which contrasts to the usually low intensity of egg parasitism reported for temperate species. These results reinforce general trends detected in recent literature reviews for more abundant and/or pest species towards the importance of natural enemies as mortality factors of herbivorous insects.

**Key words:** *Hypna*, life-table, parasitism, predation, seasonality, survivorship curve.

## INTRODUCTION

To achieve a fuller understanding of the processes and factors governing fluctuations of animal populations, an important first step consists of describing these fluctuations in the field at different time-scales, including species with different life-histories inhabiting different habitats and showing different patterns of abundance. The increase in the number of descriptive studies in different ecological contexts may lead to the detection of hidden patterns (Price, 1991) and make possible the establishment of more comprehensive and reliable generalizations (e.g. Cornell & Hawkins 1995; Hawkins et al. 1997; Cornell et al. 1998).

For temperate regions many of studies on demography of herbivorous insects are already

available, encompassing several different groups. The order Lepidoptera is relatively well represented, although many studies refer to very abundant and/or pest species (reviews in Dempster 1983; Courtney 1986; Cornell & Hawkins 1995; Hawkins et al. 1997; Cornell et al. 1998). Yet, for neotropical region, despite the increasing number of studies on population dynamics of adult butterflies (e.g. Ehrlich & Gilbert 1973; Vasconcellos-Neto 1980; Saalfeld & Araújo 1981; Quintero 1988; Freitas 1993, 1996; Ramos & Freitas 1999, Vanini et al. 2000; Freitas & Ramos 2001, Freitas et al. 2001), field investigations on immature populations of Lepidoptera in natural habitats are still scarce. This is especially true for investigations employing life-table methods, of which the few examples include the studies of Costa (1991) on the butterfly *Hypothyris*

*ninonia daeta* (Bdv.) and Caldas (1995a,b, 1996), dealing with the populational dynamics of the juvenile stages of *Anaea ryphea* (Cramer) in southeastern Brazil and in Panama. This paucity of data on mortality schedules for immature stages of neotropical Lepidoptera inhabiting natural systems is even more striking for uncommon species.

In a population study of the butterfly *Pieris virginiensis* Edwards, Cappuccino & Kareiva (1985) correctly argued that because in natural habitats most insect species are rare, the understanding of the relationships between these species and their host plants is essential to any theory of plant-insect interaction. Besides that, the greater emphasis put in studies of very abundant or of economic value species could lead us to biased generalizations (Hayes 1984; Hawkins et al. 1997). In a review on novel approaches to studies of population dynamics, Cappuccino (1995) called for more studies of rare species, in spite of the obvious difficulties of data gathering in these cases due to the low population sizes. If we want to get a broader and unbiased picture of mortality patterns for herbivorous insects, it is clear that more quantitative descriptive studies are needed.

In this study I describe the seasonal abundance fluctuation and mortality schedule for immature stages (eggs and larvae) of a population of the butterfly *Hypna clytemnestra huebneri* (Butler), a widely distributed but locally uncommon charaxine (Nymphalidae) inhabiting southeastern Brazil.

## MATERIAL AND METHODS

### Study organisms

*Hypna clytemnestra* (Butler 1866) is a medium-sized (forewing length 40-45 mm), relatively cryptic butterfly. A general description of the species can be found in Comstock (1961). In the study area females lay eggs singly on the underside of leaves of *Croton floribundus* Spreng (Euphorbiaceae), a latescent pioneering plant commonly found in the reserve. This species is also used as host plant by another Charaxinae butterfly, *Anaea ryphea*, which is very abundant in the area (Caldas 1994, 1995a) and occasionally by *A. appias* (Hübner), *A. otrere* (Hübner) and possibly *A. arginussa* (Geyer). Individuals of *C. floribundus* of varying sizes, from seedlings to saplings or trees are found in both sunny and shady areas, isolated or in patches, mostly along

the trails or on the borders of the forest. Leaves vary in size (10 to 20 cm) depending on the height of plants. During the dry season plants tend to dry out, with a reduction in the production of new leaves and in their nutritional quality (unpublished data).

Younger larvae (first to third instars) construct frass-chains (pers. obs.) which are used during the whole larval period. Larvae are sedentary, staying on the frass chains most of the time, leaving these sites only to feed during short bouts. Fifth instar larvae leave the host plants to pupate. Adults sometimes fly along sunny trails and forest gaps, but during mid-day males usually perch on sunny sites along trails, displaying territorial behavior. Like other Charaxinae, they feed on rotten fruits, carrion and feces (Young 1982; DeVries 1987).

### Study site

The study was carried out at the Reserva de Santa Genebra, located at Campinas (22° 44' S, 47° 06' W, elevation 670 m), state of São Paulo, Brazil. The reserve is a 251,7 ha patch of disturbed subtropical semideciduous moist forest, surrounded by crops of corn and soybeans, and other human infrastructures. Mean monthly temperature varies from 18°C to 29°C, with daily fluctuations of as much as 20°C from July to September (Morellato & Leitão-Filho 1995). There is a dry season from April to August, characterized by low temperatures and low precipitation, followed by a warmer and wet season, from September to May (Fig. 1).

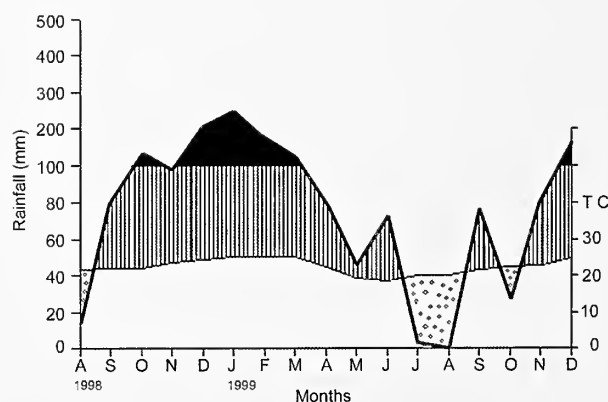


Fig. 1. Climatic diagram of the Reserva de Santa Genebra region during the study period (November 1998 - December 1999). Hatched = humid period, black = superhumid period and dotted = dry period (following Walter, 1985).

### Mortality pattern of *Hypna clytemnestra* population

To investigate the abundance and mortality pattern of *H. clytemnestra* immatures, from November 1998 to November 1999 I inspected 65 previously tagged plants of a population of *C. floribundus* for the presence of eggs and larvae. They were inspected at three-day intervals from early to mid-season, and once a week from February on. All tagged plants were distributed along the edges of a 1,160 m central trail which passes through the area. I individualized eggs and larvae through a combination of India ink markings on leaf limb and plastic rings placed around leaf petiole. These procedures permitted recordings of immature numbers and positions on each leaf and on each individual plant, making it possible to track their development and survivorship (cf. Caldas 1995a). Eggs and larvae that disappeared between two consecutive inspections were assumed as have been preyed upon.

I constructed a multiple decrement life-table (Carey 1993), which permits identification of the stages of life suffering the highest mortality as well as evaluation of the effect of different mortality factors acting simultaneously. I carried out a competing risk analysis (Carey 1993), a mathematical approximation that permits inference of the effect of one cause of mortality when another cause that occurs simultaneously is eliminated. In this method, if we have two causes of mortality, A and B,  $q_A$  is the proportion of individuals inferred to die when cause B is absent, and  $q_B$  is the proportion of individuals inferred to die if cause A is absent.  $D_A$  and  $D_B$  denote the fraction of all individuals observed to die from cause A and B, respectively. Then  $q_A$  can be found by solving the equation:

$$aq_A^2 + bq_A + c = 0$$

where

$$a = D_A \quad b = -(D_A + D_B)$$

$$c = D_B(D_A + D_B) \quad q_B = q_A D_B / D_A$$

For a detailed explanation and rationale of the method see Carey (1993: 26-29).

To evaluate the variation in adult abundance of *H. clytemnestra* I employed 12 traps (p. 35 in DeVries 1987) baited with fermented bananas, distributed as follows: four traps on the forest edges, four traps along the central trail (study trail), and four traps inside the forest. Forest edges were characterized by an abrupt transition between forest and open fields with crop vegetation. Traps on the central trail were in the middle of the forest, but in a kind of habitat looking like a gap, due to the relatively open canopy and more intense light penetration, whereas traps inside the forest were in a more humid and shady environment. Traps were hung 1.5 to 3.5 m above the ground. Each sampling consisted of opening traps around noon and closing them the following day at the same time. Captured butterflies were individually marked on the hindwing with a felt-tipped pen and then released. I took four samples each month, from March 1999 to February 2000, totaling 48 samplings.

### RESULTS

The population of *H. clytemnestra* showed a seasonal pattern of abundance fluctuation for the immature stages not seen in the adults (Fig. 2). The number of eggs and larvae increased from a very low level in late November/early December 1998 to a peak in late January/early March. This peak was then followed by a sudden reduction in number of immatures to reach the level observed at the beginning of the growing season. During the rest

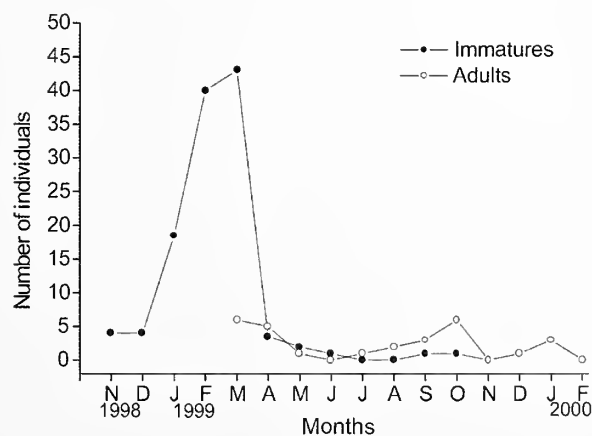


Fig. 2. Abundance fluctuation of immatures and adults of *Hypna clytemnestra* at the Reserva de Santa Genebra, Campinas, SP, from November/1998 to February/2000.

of the year just a few immatures (less than 5) or even none (in the dry season) were recorded on the host plant population.

The number of adults trapped monthly was consistently low (0 to 6), resulting in a total of only 28 individuals captured (as a comparison, at the same period 419 individuals of the abundant species *A. ryphea* were trapped). No clear peak in adult abundance was detected (Fig. 2). The captured individuals were not uniformly distributed among habitats ( $\chi^2 = 8.85$ ;  $df = 2$ ;  $p = 0.012$ ). Most were captured on the central trail (50.0%) and inside the forest (42.9%), while few were trapped on the forest edge (7.1%). Only four individuals were recaptured, with the following recorded residence times (i.e., time elapsed between capture and last recapture): 3, 18, 23 and 36 days. It should be noted that despite the extremely low abundance, adults were trapped even during the dry season months.

In all, 211 immatures were followed from egg to death. The egg stage suffered highest mortality (ca. 80.0%), attributed to both parasitism and predation (Table 1, Fig. 3). Despite this intense mortality, I did not directly observe predation on eggs or larvae of any instar during censuses, though on two occasions I observed eggs being parasitized. Although these eggs are very small, they can be readily seen with the naked eye after one gets used to the contrast between leaf surface and egg color. Several events of egg parasitism were also observed for eggs of the butterfly *A. ryphea* on the same plants.

Eggs of *H. clytemnestra* were parasitized by an Eulophidae species. Mortality of first and second instars were less intense (7.0 and 22.5%, respectively) than those of third and fourth instars (54.8 and 64.3%). Only 2.3% of the eggs reached fifth instar. Mortality of the fifth instar larvae could not be estimated since larvae in this phase of development leave the plants to pupate. All larval mortality was attributed to predation. Even if we assume mortality factors acting upon fifth instar larvae and pupae to be negligible (which is unlikely) the percentage of immatures actually reaching adulthood is less than 2.3%.

Thus, eggs and medium to large-sized larvae were the stages with greatest risk of mortality (Table 1). Even correcting for differences in stage/instar duration by considering probability of survival on a daily basis, this pattern of mortality did not change, indicating that the tendency for more intense mortality of eggs and larger larvae was not an artifact due to differences in amount of time exposed to natural enemies (see values inside parenthesis in the first column of Table 1). Therefore the intensity of mortality suffered by eggs and all larval instars considering the whole season generates a mixed survivorship curve of Types II/III, based on Deevey's (1947) general classification (Fig. 3).

The competing risk analysis for eggs showed that if predation was eliminated as a cause of mortality, egg parasitism alone would kill 58.1% of the eggs, instead of the 42.6% actually killed. If parasitism

Table 1. The multiple decrement life-table for immature stages of a population of the butterfly *Hypna clytemnestra* studied during 1998/1999 at the Reserva de Santa Genebra, Campinas, SP, Brazil.

Stage <sup>1</sup>	Number beginning	Fraction living at	Probability of death stage	Fraction of all deaths the beginning	Fraction of deaths from		Daily
					predation	parasitism	
	Kx	alx	aqx	adx <sub>T</sub>	adx1	adx2	Px
Egg (5)	211	1.0000	0.7962	0.7962	0.3697	0.4265	0.7275
Instar I (4)	43	0.2040	0.0698	0.0142	0.0142	0.0000	0.9820
Instar II (7)	40	0.1900	0.2250	0.0427	0.0427	0.0000	0.9642
Instar III (6)	31	0.1470	0.5484	0.0806	0.0806	0.0000	0.8759
Instar IV (6)	14	0.0660	0.6429	0.0427	0.0427	0.0000	0.8423
Instar V (6)	5	0.0230	-	-	-	-	-

<sup>1</sup> – Values inside parenthesis refer to the mean number of days ( $t_x$ ) spent in each stage based on field data

<sup>2</sup> – Daily probability of survival obtained as  $Px = (1 - q_x)^{(1/t_x)}$

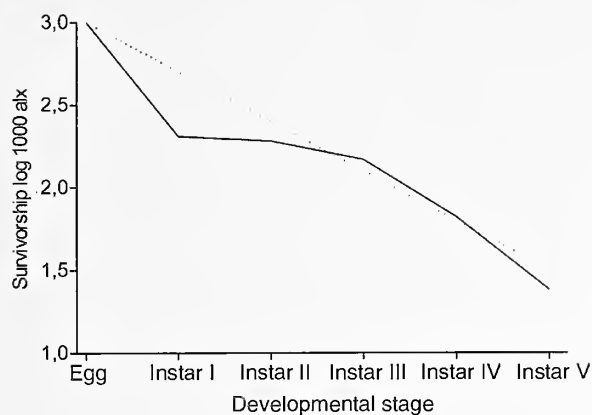


Fig 3. Survivorship curve for immatures of a population of *Hypna clytemnestra* studied for one year at the Reserva de Santa Genebra, Campinas, SP. Dashed line represents a theoretical survivorship curve with constant rate of mortality (Type II).

was eliminated, 49.0% of the eggs would have died from predation as compared to the 36.9% (Fig. 4). When mortality was considered during both egg and all larval instars, elimination of predation would increase parasitism from 42.6% to 71.7%, whereas if predators were acting alone they would be responsible for as much as 91.7% of all deaths, a significant increase over 40.0% (Fig. 4).

## DISCUSSION

*Hypna clytemnestra* immatures showed a marked seasonal fluctuation in abundance during the year, a pattern not followed by the adult stage. The increase in immature number was well synchronized with the increase in rainfall in the wet season. During this time there is an increase in food availability, since a greater number of new leaves, of better nutritional quality are produced by host plants (unpublished data). A likely scenario is that the arrival of summer also results in hotter and longer days and larvae from eggs hatching at this time will develop in a warmer environment and might be feeding on more abundant and better quality leaves. These simultaneous changes in adult and larval environment will act in combination, favoring a faster larval development, shortening generation time and leading to a rapid increase in the butterfly population.

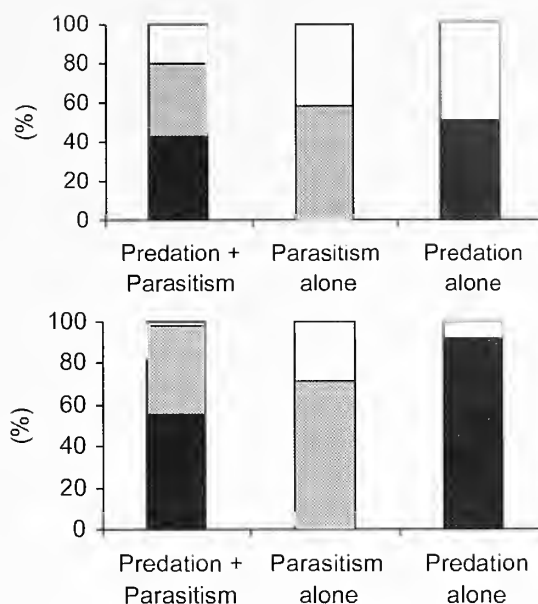


Fig. 4. Intensity of immature mortality inferred from the competing risk analysis applied for eggs alone (A) and for eggs and larval instars combined (B). Left bar shows the original life-table results, middle bar shows the impact of parasitism if predators were not acting and right bar shows the impact of predation in the absence of parasitism.

On the other hand, the proximity of the dry season leads to host plant deterioration (i.e. accumulated leaf damage, reduction in water and nitrogen content), which may sometimes result in a reduction in reproductive activity in seasonal habitats (Jones & Rienks 1987; Braby 1995). In addition to that, the increase in immature density in the middle of season may increase the encounter and attack rates on eggs and larvae by parasitoids and predators, which will probably buffer the population increase. The rate of parasitism for fourth instar larvae of *A. ryphea* feeding on the same host plant seemed to increase in response to increase in larval density (Caldas 1996). Other examples of positive density dependence action of parasitoids and predators on herbivorous insects can be found in Stiling (1987, 1988).

In addition to the positive effect mediated by improved host plant quality, a possible negative effect of heavy rainfall on immatures of *Hypna* is the mortality of eggs and larvae by dislodging from leaves (e.g. Blau 1980; Courtney & Duggan 1983). Although such effect was reported by Caldas (1995a) for larvae of the butterfly *A. ryphea* using the same host plant in the area, it was not observed for larvae of *Hypna*.

Young (1981) evaluated the seasonal distribution of two species of Charaxinae in a dry forest of Costa Rica, *Anaea morvus* Boisduvali and *Anaea (Zaretis) itys* Cramer. For both species, immatures and adults were abundant mainly in the wet season, being rare or even absent during dry season. He detected a low level of larval parasitism and suggested that the seasonal pattern of abundance of *Anaea* populations was a response to a reduction in food quantity and quality. A marked seasonality in immature abundance was also reported for the butterfly *H. ninonia daeta*, which reached high densities at the end of the wet season in a forest patch in southern Brazil (Costa 1991). Host plant abundance and quality was suggested as of primary importance in the dynamics of *H. ninonia daeta*. The correlation between fluctuation of insect abundance and seasonality of rainfall and food availability in tropical habitats has been reported previously by Wolda (1978a, b), but despite the good match between rainfall and population fluctuation, it may be that rainfall pattern is not the main factor causing the increase in immature population of insects (Wolda 1988).

The general mortality pattern described here consists of higher mortality of eggs and late larval instars and high survival of small larvae. However, in a population of *A. ryphea* studied in the same site and feeding on the same host plant, first instar larvae was the stage showing higher mortality during the season, which was attributed mostly to predation and to intense rain (Caldas 1995a, 1996). Nevertheless, in these previous studies mortality of eggs was not quantified. On the other hand, the mortality pattern reported for immatures of *H. ninonia* was similar to that found for *H. clytemnestra*, with more intense mortality suffered by eggs and final instars (Costa 1991).

In temperate regions, population studies including the construction of life tables for immature stages were carried out for some pierid species (e.g. Courtney & Duggan 1983; Cappuccino & Kareiva 1985; review in Courtney 1986). A different mortality pattern was reported for *Anthocharis cardamines* (L.) in Britain, with a relatively low egg mortality and low survival of larvae in early instars (Courtney & Duggan 1983). The extremely low egg parasitism is opposed to the high parasitism rate found for *Hypna*. For the rare *Pieris virginiensis* larval survival was similar to those reported for other

more abundant pierids, with less intense mortality during the egg stage and final instars and a higher survival of the initial instars (Courtney 1986). Some other studies showing similar results are those of Courtney (1981) and Hayes (1981) - Pieridae, and Blau (1980) and Rausher (1980) - Papilionidae, while Watanabe (1981) and Feeny et al. (1985) found more constant rates of mortality from eggs to late instars for species of *Papilio*, characterizing a Type II survivorship curve.

The competing risk analyses for *Hypna* revealed that for the egg stage both predation and parasitism are equally important as mortality factors, since the elimination of each one of them leads to a similar compensation (increase) in the intensity of mortality caused by the other. When both eggs and larvae are considered, predators seem to be playing a more important role, since predation would compensate better for the absence of parasitism than vice-versa.

In most published studies mortality was caused by different factors at different stages/instars (see review in Dempster 1984). Eggs can fail to hatch due to apparent infertility, suffer from disease and bacterial attack, be dislodged by rain, die from dissection or other unknown physiological causes, be parasitized or predated (Baker 1970; Parker 1970; Young & Moffet 1979; Hayes 1981; Watanabe 1981; Courtney & Duggan 1983; Caldas 1995a, 1996). Small larvae can be dislodged by rain, fail to establish in the host plant due to physical or chemical barriers, be parasitized or predated by small arthropods (Baker 1970; Blau 1980; Courtney 1981; Hayes 1981; Watanabe 1981; Courtney & Duggan 1983; Caldas 1995a; Ohsaki & Sato 1994, 1999), while larger larvae tend to be predated by vertebrates, especially birds (e.g. Pollard 1979; Watanabe 1981).

In this study the only cause of mortality detected directly was parasitism of eggs. Parasitized eggs change colour into metallic gray. Predation can only be inferred by the disappearance of eggs and larvae. Despite no observation of a predation event upon *Hypna* immatures, possible invertebrate predators of eggs and small larvae in the area would be ants, spiders and wasps, the first two groups being relatively abundant on *C. floribundus*. Evidence supporting this are observations of ants preying upon eggs (J. M. Queiroz, pers. com.) and of a jumping spider (Salticidae) preying upon a second instar larvae of *A. ryphea*. The direct observations

of predation on immatures of *A. ryphea* were probably favored by its higher abundance, and there is no reason to believe that these same predators would not kill immatures of *Hypna* as well. Potential predators of large larvae are wasps and birds.

Attacks by predators, parasitoids and pathogens were the most frequently cited sources of mortality (48%) for immatures of herbivorous insects in 530 published life-tables evaluated by Cornell and Hawkins (1995), which included some studies on Lepidoptera. In a more quantitative analysis of 83 life tables for 78 herbivorous insect species, predators appeared as the dominant natural enemy (as compared to parasitoids and pathogens) of post-egg stages in the tropics and subtropics, whereas parasitoids are dominant in temperate zone (Hawkins et al. 1997). For the population of *H. clytemnestra* investigated, inhabiting a tropical and seasonal environment, natural enemies emerged as the main mortality factors. Considering all stages and instars, predators played a major role, agreeing with the pattern suggested for the tropics in the above review. For the egg stage, death rate was equally due to parasitoids and predators, but the former killed a higher fraction of eggs of *Hypna* than is usual in species of temperate regions.

Regarding habitat use, adults of *Hypna* seem to use equally well both shady, inside forest and gap-like habitats, avoiding to some degree more open habitats such as forest edges. With respect to the low abundance of adults, it is worth noting that I started trapping only in March, and maybe a slight peak in adult abundance in the previous months, namely January and February, would have been detected if I had used traps at that time. On the other hand, despite the decrease of immature numbers in March, the population was still "large". Therefore, I would expect that captures in the following two months would reflect this through the trapping of a higher number of adults, that did not occur. Also interesting was the presence of adults through all the year, even during the drier and colder months, and the low number of individuals trapped in January and February, 2000.

For the rare *Pieris virginiensis* in temperate regions, unfavorable climatic conditions for flying and laying eggs, difficulty in finding the host plant hidden by neighboring vegetation, mortality caused by starvation and predation when locating secondary host plants to complete development, and the

inability of adults to cross open fields and colonize new adequate habitats all acted to shape the populations at low densities (Cappuccino & Kareiva 1985). Except for unfavorable climatic conditions, most of the cited factors do not apply to *H. clytemnestra*. Its low abundance, even during the middle of the wet season when individuals are supposed to have been the best environmental conditions, seems to be shaped mainly by the intense mortality suffered by the immature stages due to the action of natural enemies, namely high parasitism and predation upon eggs and predation upon larvae.

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# Habitat utilization by ovipositing females and larvae in an endangered population of the moth *Dysauxes ancilla* (Lepidoptera: Ctenuchidae)

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**Abstract.** Habitat utilization by ovipositing females and larvae was studied in the endangered Swedish population of the moth *Dysauxes ancilla*. Ovipositing females and larvae were found to have specific habitat requirements, being restricted to edge zones facing south and to an ancient shore line facing southwest. The dependence of these microhabitats can be ascribed to two factors. First, larvae occur where there are both a high abundance of food plants and well developed layers of *Quercus robur* litter. Second, being on the northern edge of the species' distribution the need for warm microclimatic conditions is strong. These conditions were only met in the preferred microhabitats. Among the larval food plants *Hieracium pilosella* was clearly indicated to be the most important species. The habitat preferred by larvae and ovipositing females constitutes a transient successional stage. This highlights the need of maintaining a continuity of the preferred microhabitats and prevent them from overgrowth.

**Key words:** conservation, food plant, larvae, microclimate, oviposition, Sweden

## INTRODUCTION

Lepidopteran populations have declined in many parts of the world during the last century (Pollard & Eversham 1995). As a consequence, the interest in conservation work of Lepidopterans has increased. Several studies have shown that ovipositing females and preimaginal stages of Lepidoptera usually have a much narrower niche range than was formerly believed (Thomas 1984, New et al. 1995). This seems to be even more apparent in endangered species (e.g. Warren 1987), which require specific microclimatic conditions or host plants growing only at certain microsites (Thomas et al. 1986, Warren 1993, 1995). In early conservation attempts habitats were often set aside without identifying the needs of the endangered species in focus. This caused the extinction of several butterfly species in nature reserves in Great Britain (Thomas 1991). Thus, detailed knowledge of habitat requirements of ovipositing females and larvae is crucial for a successful conservation of

endangered Lepidopterans.

The Swedish population of *Dysauxes ancilla* (Linné 1767) (Lepidoptera: Ctenuchidae) is currently restricted to a small area (4 ha) near Beijershamn on the Baltic island of Öland (Betzholtz & Lindeborg 1996). The population constitutes an isolated northern outpost, separated from the species' main distribution area in central and eastern Europe (Betzholtz 2000). The preferred habitat of the Swedish population consists of the edge zones of dry meadows with short vegetation and solitary junipers and oaks. An ancient shore line, 6000 years old, from the Litorina era is also part of the breeding habitat. This shore line is now 400 m from, and 5 m above, the sea level due to the postglacial landupheaval. It is 5 m wide and with a slope of 15° towards southwest. The vegetation of the shore line is an open short grass community.

During the last few decades overgrowth has led to a reduction of the species' habitat. As a result *D. ancilla* has disappeared from a large part of its former area and the population size was estimated

at approximately 2000 individuals in 1993 (Betzholtz & Lindeborg 1996). The species is listed as critically endangered in Sweden (Gärdenfors 2000).

In Sweden the species is univoltine. Adult moths fly between late June and middle July, and are active mainly during daytime and at dusk. The species hibernates in the larval stage. An experimental study of food plant suitability for larvae of *D. ancilla* (P-E Betzholtz, unpublished manuscript) showed that four plant species supported full development to the adult moth, as did a mixed diet composed of all plant species included in the experiment. According to survival, larval development time and female imago weight there was a significant suitability order between these plants. *Calluna vulgaris* (Ericaceae) and the mixed diet were equally suitable and preferred over *Hieracium pilosella* (Asteraceae) which was preferred over *Thymus serpyllum* (Lamiaceae) which was preferred over *Brachytecium* sp. (Brachytheciaceae).

The objective of this study is to determine the habitat and food plant preference by ovipositing females and larvae of *D. ancilla* in the field.

## METHODS

### Oviposition

Ovipositing females of *D. ancilla* sit low in the vegetation and drop their eggs to the ground, without attaching them to any substrate (P-E Betzholtz, unpublished manuscript). Females of some other species with polyphagous grazing larvae oviposit near but not on the preferred food plant (Thompson 1988, Bergman 1999). Therefore, I consider every oviposition as a choice of food plant or a choice of microclimatic condition.

I actively looked for ovipositing females in the habitat and immediately after oviposition the exact position of the oviposition site was recorded. I then measured the orientation to, and distance from, the nearest edge zone or the ancient shore line base for each oviposition. All plant species within 1 m<sup>2</sup> around the oviposition site were recorded.

### Larvae

The edge zones and the ancient shore line, where females had been observed ovipositing, were searched for larvae along 15 randomly selected transects at three different occasions; late

September, early April and early June. The transects, 1 m wide and perpendicular to the edge zones, extended 5 m out on the open meadow and 5 m in under the tree canopy. Five transects were orientated towards south, five towards east and five towards west. In edge zones facing north I never observed any ovipositions. At the ancient shore line I had nine transects, perpendicular to the shore line and extending 5 m out from the base and top respectively. Larvae are partly active during night (P-E Betzholtz, personal observation). Therefore, one third of the transects were searched at night with a halogene spotlight.

Along the transects all larvae found were recorded and I noted orientation to, and distance from, the nearest edge zone or the ancient shore line base. All plant species within 1 m<sup>2</sup> around the larval finding were recorded.

All larvae found in the field were brought to the laboratory and reared. In this way information of the parasitoid pressure on *D. ancilla* was obtained.

### Vegetation

To find out if there was a preference for certain plant species among ovipositing females or among larvae, I did a vegetational analysis. I randomly chose 50 1-m<sup>2</sup> plots in the dry meadows of the breeding habitat and recorded all occurring plant species including *Quercus robur* litter within the plots. I calculated the relative frequencies for each species by dividing the number of plots where the species occurred by the total number of plots analyzed. I used data from the oviposition and larval plots described above to calculate the relative frequency of all plants found in these plots. Then, I calculated the deviations between random plots, and oviposition and larval plots, for each plant species. The deviations were calculated as the relative frequency in oviposition or larval plots minus the relative frequency in the random plots. A positive deviation means a higher occurrence of the plant species at the oviposition or larval sites, a negative deviation a higher occurrence at the random sites.

*C. vulgaris*, *H. pilosella*, *T. serpyllum* and *Brachytecium* sp. supported full development to the adult moth in an experimental study (P-E Betzholtz, unpublished manuscript). These species are hereafter referred to as food plants. In order to compare their distribution across the edge zones, with the ovipositions and larval findings, I

determined their occurrence along the transects described above for the larvae. Along the transects I recorded the presence or absence of these four food plants, with all four treated as a group, in continuous 1 m<sup>2</sup> plots. I also recorded the presence or absence of *Q. robur* litter. The distribution of food plants and *Q. robur* litter was analyzed with a logistic model with distance from the canopy edge as regressor (Sokal & Rohlf 1995).

## RESULTS

Thirty ovipositions were observed and 40 larvae were found in the field. Ovipositing females and larvae showed a strong affinity for the edge zones (ovipositions:  $n=23$ ; larvae:  $n=26$ ) and the ancient shore line (ovipositions:  $n=6$ ; larvae:  $n=14$ ), and only one female oviposited in a meadow far from any edge zone. 83% of the ovipositions and 70% of the larvae were found within 1 m from the canopy edge (Fig. 1). Edge zones facing south, that is orientated east-west, were preferred both by ovipositing females and larvae (ovipositions:  $X^2=23.7$ ,  $df=3$ ,  $p<0.001$ ; larvae:  $X^2=29.8$ ,  $df=2$ ,  $p<0.001$ ; Fig. 2). At the ancient shore line all six ovipositions were found within 2 m, and all larvae within 3 m, from the base.

All larvae were encountered during the survey in early June, and all except two were recorded

during daytime. No parasitoids emerged from the larvae or pupae reared. All larvae were found on the ground and only where a layer of *Q. robur* litter was present. Furthermore six of the larvae were foraging, all on *Q. robur* litter, when encountered. To find out if the distribution of *Q. robur* litter was important to the larvae, I divided the 1 m<sup>2</sup> plots where larvae were found into three classes according to percentage cover of *Q. robur* litter; I: 0–25% coverage, II: 25–50% coverage, III: >50% coverage. Since most larvae were found within 1 m from the canopy edge facing south, randomly chosen plots in this microhabitat were also analyzed for percentage cover of *Q. robur* litter. The distributions (larvae: I=4, II=11, III=25; random: I=12, II=14, III=14) differed significantly ( $X^2=14.5$ ,  $df=2$ ,  $p<0.001$ ), showing that larvae occurred in plots with well developed layers of *Q. robur* litter.

The frequencies of plant species at the oviposition, larvae and random sites are shown in Table 1. The plant frequency at the random sites differed significantly from those at the oviposition sites ( $X^2=36.4$ ,  $df=21$ ,  $p<0.05$ ) and from those at the sites where larvae were found ( $X^2=37.8$ ,  $df=21$ ,  $p<0.05$ ). The difference was, in both cases, mainly due to the distribution of *Q. robur* litter (Fig. 3). Further, among the food plants of *D. ancilla* larvae only *H. pilosella* was frequent in the larval plots and in fact was more frequent there than in the habitat

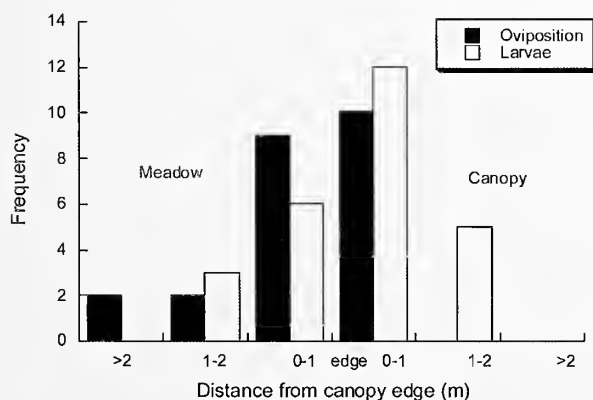


Fig. 1. Number of ovipositions ( $n=23$ ) and findings of larvae ( $n=26$ ) in *Dysauxes ancilla* at different distances from the nearest canopy edge.

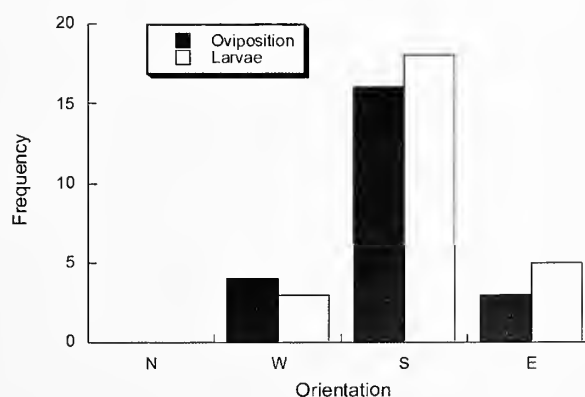


Fig. 2. Number of ovipositions ( $n=23$ ) and findings of larvae ( $n=26$ ) in *D. ancilla* in relation to the orientation of edge zones. Orientation denotes the direction that the edge zone is facing.

Table 1. Frequencies of plant species in 1 m<sup>2</sup> plots at the random sites (n=50), at the oviposition sites (n=30) and at the sites of larval findings (n=40) of *Dysauxes ancilla*. Plant species with five or less occurrences at random sites are excluded from the table. The plant species are sorted after decreasing frequency at the random sites and the species number refers to Fig. 3.

Plant	Random	Oviposition	Larvae
1. <i>Achillea millefolium</i>	35	16	27
2. <i>Galium verum</i>	33	22	19
3. <i>Deschampsia flexuosa</i>	25	19	27
4. <i>Hieracium pilosella</i>	20	13	26
5. <i>Festuca ovina</i>	19	9	13
6. <i>Veronica officinalis</i>	19	2	8
7. <i>Plantago lanceolata</i>	18	4	15
8. <i>Quercus robur</i> , litter	15	28	40
9. <i>Arrhenatherum elatius</i>	14	7	11
10. <i>Brachytecium</i> sp.	13	7	7
11. <i>Hypericum perforatum</i>	13	2	11
12. <i>Lychnis viscaria</i>	12	3	10
13. <i>Melampyrum pratense</i>	12	9	14
14. <i>Rumex acetosa</i>	11	1	4
15. <i>Calluna vulgaris</i>	9	6	3
16. <i>Allium oleraceum</i>	8	4	3
17. <i>Phleum pratense</i>	8	5	4
18. <i>Potentilla argentea</i>	7	5	7
19. <i>Rumex acetosella</i>	7	7	4
20. <i>Sedum acre</i>	6	2	1
21. <i>Sedum telephium</i>	6	1	7
22. <i>Thymus serpyllum</i>	6	2	3

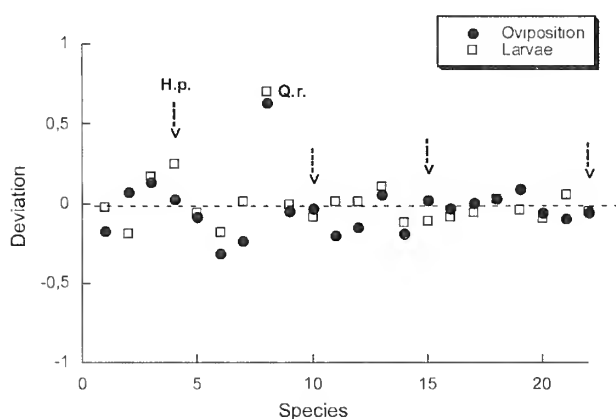


Fig. 3. Deviations in plant species frequency between random sites and sites of ovipositions and larval findings of *D. ancilla*. A positive deviation means that a plant has a higher relative frequency in the oviposition or larvae sites than in the random ones. It is clear that *Quercus robur* litter (Q.r.) has a much higher occurrence in oviposition and larvae sites than in random ones. Among the food plants of *D. ancilla*, denoted by arrows, *H. pilosella* (H.p.) is clearly indicated to be the most important species to the larvae. The species number refers to Table 1.

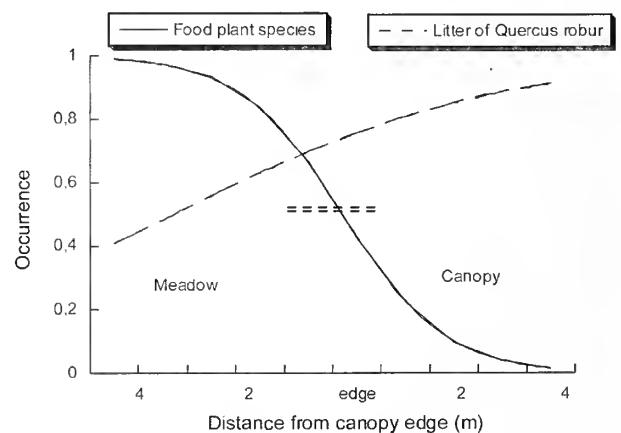


Fig. 4. Logistic regression models of the occurrences of *Q. robur* litter and food plant species for larvae of *D. ancilla* along 15 transects perpendicular to the edge zones. A high frequency of both *Q. robur* litter and food plants is only found in a narrow zone close to the canopy edge, where also larvae of *D. ancilla* were found (denoted by == ==).

as a whole.

The plant frequency at the oviposition sites did not differ significantly from the one where larvae were found ( $X^2=25.3$ ,  $df=21$ ,  $p=0.236$ ). However, the mean number ( $\pm$  SD) of plant species was significantly higher at the sites where larvae were found ( $9.3 \pm 2.7$ ) than at the oviposition sites ( $7.8 \pm 2.8$ ) ( $t=25.9$ ,  $df=69$ ,  $p<0.001$ ).

The occurrence of food plants was significantly higher out on the open meadow than under the canopy (Wald's  $X^2=42.4$ ,  $df=1$ ,  $p<0.001$ , logistic regression; Fig. 4), while *Q. robur* litter was significantly more abundant under the canopy than out on the open meadow (Wald's  $X^2=17.2$ ,  $df=1$ ,  $p<0.001$ , logistic regression).

## DISCUSSION

The null hypotheses were rejected, showing that ovipositing females and larvae in the Swedish population of *D. ancilla* have specific habitat requirements. Both stages were restricted to edge zones facing south and to the base of an ancient shore line facing southwest. Several recent studies of endangered Lepidoptera also showed a very high specificity in niche range by the ovipositing females and the preimaginal stages (Thomas 1984, New et al. 1995). Two important factors determining niche range are food plant suitability and microclimatic conditions (Chew & Robbins 1984). Food plants of *D. ancilla* grew in all parts of the studied habitat but all ovipositions except one, and all the larvae, were found in the microhabitats described above. These microhabitats probably provide temperatures high enough since their orientation ensure a high solar insolation. This is consistent with the findings of Bourn and Thomas (1993) who pointed out that the abundance of food plants growing under the right microclimate conditions is more important to the preimaginal stages than the total abundance of food plants.

The oviposition behavior of *D. ancilla* differs from the majority of Lepidoptera (Chew & Robbins 1984) in that eggs are dropped to the ground and not attached to a plant substrate. Thompson and Pellmyr (1991) stated that such a behavior was associated with polyphagy in the larvae. Further, Porter (1992) pointed out that an apparent non-selectivity in female food plant choice can be related

to a stronger dependency on microclimate conditions. The oviposition behaviour of *D. ancilla* could therefore be a strategy for occasions when high insolation and extreme drought only provide high humidity near the ground. High humidity has been shown an important factor in egg hatchability, especially in species which drop their eggs to the ground (Karlsson & Wiklund 1985, Bergman 1999).

In a univoltine species as *D. ancilla*, with hibernation in the larval stage, high ground temperatures in spring provide an early start of larval activity (Dugdale 1996) with an increased development rate and survival in larvae (Thomas 1985). Larvae of *D. ancilla* could also gain an extra advantage if they use the canopy edge in a flexible manner under extreme conditions. When weather is warm and dry the larvae may crawl into shady areas under the canopy, while remaining in the warmer edge zones during colder conditions.

All larvae were found in well developed layers of *Q. robur* litter. However, in a rearing experiment this substrate as a single food source did not support development to imago (P-E Betzholtz, unpublished manuscript). This indicates that *Q. robur* litter is not primarily used as a food substrate. Instead, I suggest that larvae of *D. ancilla* are dependent on a certain amount of humidity, as shown in other larvae of Lepidoptera living in the litter layer (Dugdale 1996).

The dependence on specific microclimatic conditions is pronounced in Lepidoptera living in the northern part of their distribution area (Warren 1989, Thomas 1993). The Swedish population of *D. ancilla* constitutes an isolated northern edge population. Thus, my results indicate that ovipositing females and larvae have a strong dependence of warm microhabitats with sufficient humidity, conditions only met in sun-exposed edges and slopes with *Q. robur* litter.

Among the food plants, according to the laboratory rearing experiment of *D. ancilla*, only *H. pilosella* had a higher relative frequency in larval plots than in random ones indicating it as the most important larval food plant. In addition, larvae of *D. ancilla* are mobile and starvation tolerant, surviving more than a week without food (P-E Betzholtz, unpublished data). Therefore, larvae should have no difficulty searching for foodplants growing in the proximity of the litter while spending most of the other time hiding in the litter. Dethier



(1987) observed the same pattern for *Diacrisia virinica* (Arctiidae), a polyphagous species taxonomically related to *D. ancilla*. High abundance of both food plants and *Q. robur* litter was only found in the narrow zone along the canopy edge and along the shore line base providing a likely explanation for occurrence of the larvae in these microhabitats.

There were no indication that *C. vulgaris*, the most suitable plant in the laboratory rearing experiment, was included in the larval diet. *C. vulgaris* had a lower relative frequency in the larval plots than in the random ones, and indeed only occurred in 8% of the larval plots. Other things being equal, it seems that the most suitable food plant should be included in the diet. Hesjedal (1983) showed that polyphagous species have a faster development, a higher weight gain and a higher reproductive capacity when feeding on the most suitable food plant. Furthermore, low correlation of larval preference and performance indicates that factors other than food plant characteristics influence larval performance (Wiklund 1982, Thompson 1988), especially in species where larvae feed as grazers and move among several plants during development (Thompson & Pellmyr 1991). One explanation for the poor correspondence in *D. ancilla* could be selection for enemy-free space (Gilbert & Singer 1975). If the selection pressure on larvae feeding on the suitable food plant species is very high due to parasitism (Bernays & Graham 1988), predation (Rausher 1979, Warrington 1985) or competition, then species lower in suitability rank may be used. In this respect it is of interest to note that there was no parasitism on larvae of *D. ancilla* found in the litter layer, and that *H. pilosella*, clearly the most utilized plant, was the second most suitable species in the laboratory rearing experiment (P-E Betzholtz, unpublished data). Ctenuchid species are attacked by ichneumonids (Curl & Burbutis 1978), and the taxonomically related *Paidia murina* (Arctiidae), is attacked by at least four different parasitoids (García-Barros 1984).

Another explanation for the poor correspondence is that *C. vulgaris* is rare in, or grows in unfavorable parts of, the breeding habitat of *D. ancilla*. However, the frequency of the perennial *C. vulgaris* is stable and grows along the preferred edge zones and on the ancient shoreline. Given the results of this study it is not possible to distinguish

among the above explanations for avoidance of *C. vulgaris*.

I conclude that the habitat utilization of *D. ancilla* depends on choices made both by ovipositing females and in dispersing larvae. First, ovipositing females choose warm microclimatic sites with a high abundance of food plants and well developed layers of *Q. robur* litter. These conditions are only met along the edge zones facing south and at the base of the shore line facing southwest. Second, the free-living larvae search for, and stay on, suitable individual plants when foraging and spend most of their time concealed from predators and parasitoids in the litter. The ability of larvae to endure starvation permit them to disperse and find suitable microhabitats. Finally, *H. pilosella* is the most important food plant species for larvae of *D. ancilla* in this edge-of-range population.

## CONSERVATION IMPLICATIONS

Ovipositing females and larvae of *D. ancilla* were found to have specific habitat requirements both with respect to warm microhabitats and vegetation composition. The preferred microhabitats, edge zones of the dry meadows facing south and the ancient shore line, constitutes a successional stage that in the long run will be overgrown rendering the habitat unsuitable to *D. ancilla*.

During the last decades the area of the breeding habitat in Beijershamn has decreased by overgrowth (Betzholtz & Lindeborg 1996). Currently there are no other suitable habitats in the surrounding matrix. Hence, a key factor for short-term management to provide future survival of *D. ancilla* is to maintain these specific microhabitats. This could be achieved either by manual clearings at regular intervals or by selective grazing to prevent overgrowth. It is important to maintain both the warm edge zones and the plant community of the dry meadows containing *H. pilosella*. If the vegetation height of the dry meadows is allowed to increase, this will be harmful to the larval food plants of *D. ancilla*. However, some recently overgrown areas situated adjacent to the current breeding habitat still have a suitable flora. I suggest that these areas be improved by clearing of trees and shrubs. Several old oak trees remain in this area. If some of the oaks are spared,

there will also be suitable edge zones in the cleared area. The long-term survival of *D. ancilla* is dependent on the maintenance of suitable edge zones and openness which enhances the plant community of the dry meadows. Therefore, it is important to include the vegetation dynamics of the habitat, and the regeneration of future edge zones, into any management plan. The possibilities for creation of new suitable habitats in the surrounding matrix should also be considered. The Swedish population of *D. ancilla* thus far remains genetically intact (Betzholtz 2000), hence the population could be used for an expansion in the adjacent area.

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# Mass rearing the endangered Palos Verdes blue butterfly (*Glaucopsyche lygdamus palosverdesensis*: Lycaenidae)

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**Abstract:** Mass rearing of the endangered lycaenid *Glaucopsyche lygdamus palosverdesensis* (Palos Verdes blue butterfly) is described. Numerous problems were encountered in our attempts to predictably produce a large stock population both as insurance against extinction and for re-introduction to sites where the species has been extirpated. We describe our approaches to mass rearing with discussion of all aspects of life history, difficulties with parasitoids and predators, cage design, and artificial diet use. Both cylindrical cages placed over individual potted plants and outdoor tent cages were successful in providing conditions where captive individuals would mate without intervention, transcending previous limits posed by hand pairing. From a small initial stock, we produced between 168 and 968 pupae each season. Highest losses were experienced in first instar, with later losses from microsporidian infection. Predation during pupation was also significant in semi-natural confined conditions. The effort has been in progress for eight years and is continuing.

**Key Words:** *Glaucopsyche lygdamus palosverdesensis*, Palos Verdes blue butterfly, mass rearing, artificial diet, mass selection, predators and parasitoids, cage design.

## INTRODUCTION

The scientific literature reports few attempts to mass rear butterflies, that is, to produce large quantities of a species for experimental, or, more recently, conservation purposes (Mattoon et al. 1971; Lees 1989; Herms et al. 1996). Production under less controlled conditions has been explored through butterfly ranching as a tool for conservation and sustainable harvest of tropical butterfly species (Parsons 1984; New 1994). Methods for mass rearing of butterflies in more controlled conditions have not been thoroughly described, notwithstanding well developed methods to mass produce several moth species for economic purposes as sterile control programs, and success producing many insect parasitoids for biocontrol (Parrella et al. 1992; Hassan 1993). In the latter cases production of millions of individuals per day have been achieved (King & Leppla 1984; Thompson 1999).

Development of artificial diets allowed these production levels (Singh & Moore 1985; Anderson & Leppla 1992), but only for species that mate rapidly in confined spaces.

Because life histories of many butterfly species are relatively well known, mass rearing would seem relatively simple given adequate funding resources. However, because very few butterflies have recognized economic value, few incentives exist to develop such methodology (Lees 1989; Samways 1990). With advent of the U.S. Endangered Species Act, captive pro-pagation and mass rearing may now be heuristic endeavors. Already many programs have been implemented, several at the cost of many millions of dollars, to rescue nearly extinct animal species. Conservation agencies devoted extensive resources to captive rearing of vertebrates, most famously California condor and black-footed ferret (Meffe & Carroll 1997), while some listed butterflies have also been reared in captivity (Herms et al. 1996).

Although all butterflies are amenable to captive rearing, including the usually difficult problem of inducing mating, large scale production is not in place. Butterfly farming for butterfly houses, production of specimens for release at special events, and educational use for hands-on student observation of metamorphosis has increased (New 1994). Although there are no quantitative estimates of production rates, these are labor intensive and fall far short of constituting an industrial, predictive process.

All groups of butterflies have been captive reared, at least from egg to adult, with most efforts depending upon natural foodplants. The limiting factor to continuous rearing of many species has been inducement of mating, for which hand pairing was developed (Clarke & Sheppard 1956). The technique is tedious, impractical for mass rearing, and likely results in unwanted artificial selection. Below we describe methods that breach the limits of hand pairing for an endangered butterfly species, the Palos Verdes blue butterfly (*Glaucopsyche lygdamus palosverdesensis*).

Following rediscovery of the Palos Verdes blue butterfly in 1994 at the Defense Fuel Support Point (DFSP), San Pedro, California, a captive propagation effort was begun (Mattoni 1994). It was immediately apparent that this sole population of the species was in danger of extinction from stochastic factors; the wild population was only a few hundred (Mattoni 1994). The rearing program has operated since 1995, and this paper outlines the methods and results for captive rearing through the 2002 season. Unless specifically stated, the techniques used, results, and problems were from 2002. The rearing project has been conducted with a permit from the U.S. Fish and Wildlife Service (USFWS; Mattoni: TE-807303-4). As such, the program initially followed methods recommended by the USFWS for endangered lycaenid butterflies that previously had been developed by Mattoni (1988).

The three objectives of the captive breeding program for the Palos Verdes blue butterfly were: 1) to provide insurance against stochastic loss of the sole and diminished population of this species; 2) to increase size of this only known population of the insect at DFSP and; 3) to produce sufficient numbers of individuals to reintroduce the species onto revegetated sites from which it has been

extirpated across the Palos Verdes peninsula. Thus far, the program has achieved all three goals — we have maintained a captive population since 1995, we established new populations of the butterfly from captive stock at DFSP, and we attempted a reintroduction in the former range of the species with captive reared stock.

This paper reports on the rearing process itself, details about reintroductions on and off the DFSP site are reported elsewhere (Mattoni 2002).

## GENETIC CONSIDERATIONS

### Mass selection

Under any breeding system changes in gene frequency will occur across generations by either natural or artificial selection, or random sampling (genetic drift) (Mackauer 1972; Mackauer 1976). The changes are inevitable because the environment of the breeding system will not be the same as the environment of the natural habitat. Both pre- and post-zygotic selection will occur whether detectable or not. If the breeding system is designed to save and randomly mate every individual, at some point more individuals are produced than resources can maintain. The goal of any captive breeding system for conservation is to retain the substantial hidden genetic variation within natural populations (see Dimock & Mattoni 1986), and to reduce drift and selection on the population so that the resulting individuals maintain their adaptation to natural conditions (see Nunney 2002).

The captive propagation program then must establish the end use of stocks, a decision that must be taken in view of the relationship of  $N_c$  of the natural population and its ecological and genetic circumstances. Questions to be considered are whether the captive population should be maintained in parallel using only the original captures, whether new wild stock be introduced into the captive stock, or whether there be regular releases of captives while simultaneously introducing new wilds, or not, into the captive stock. Under any scenario, however, ease of consistent production of large numbers of individuals remains the key consideration. Until this objective is reached — and it has not been — other issues are moot.

We refer to mass rearing simply as the production of large numbers of individuals from a small initial

stock, then expanded by randomly mating all offspring of the following generations. Mass selection refers to the emphasis on random mating. This does not imply that selection is not occurring, but rather that as little as possible influence is exerted on the choice of mates within the system, or on the survival of any given individual. Accordingly as adults eclose, they are accumulated for one to two days and then either mated as two pairs set into small (gallon) cages, or more than two pairs into large (tent) cages. If fertile, eggs are laid on foodplant and larvae allowed to develop. The concept is that some choice of mate is provided and all offspring are given equal opportunity to develop. Thus selection is a mass phenomenon with minimal manipulative intrusion.

Others have suggested that naturalistic conditions may be used to reduce the selective effect of laboratory conditions on captive stock of insects (Boller 1972; Mackauer 1976). The use of outdoor tents is consistent with this suggestion, and is important within a conservation context where it is essential that the reared stock retain its adaptation to natural conditions (Mackauer 1976). Bryant and others (Bryant et al. 1999) by contrast emphasize the maintenance of fitness in captive populations by selectively mating high performance breeders or by high frequency immigration. Whatever approach is taken must depend on overall management goals and objectives. We believe mass selection combined with periodic immigration of wild stock is preferable at DFSP.

### Breeding Stock

The adult stock for 2002 was almost entirely derived from progeny of five wild females originally confined in 1999. The only new genetic resources were 12 pupae from four wild females taken and confined in 2000. The six adults that eclosed from the wild stock were randomly mixed with 692 year 2000 adult offspring used for the 2001 breeding population. The resultant 2001 pupal population in turn produced 150 adults. These were combined with 17 adults from the carryover pupae from 2000 for the 2002 breeding stock.

Indeed every captive adult was involved in the breeding system after year 1999. Although most production was from a few cages (e.g., of the 150 adults from 2001, 72% were derived from two tent cages) this clustering may not have had a bottleneck

effect given the small initial stock. Even assuming operation of some selection process, the likelihood of increases in homozygosity and/or loss of alleles cannot be significant given the numbers produced in 2002 (165) relative to the original five females from 1999.

### MATERIALS

After experimenting with a miscellany of cage configurations, we adopted two types of confinement chambers for general use after year 2000. The first, "gallon cages," consisted of either a cylinder of clear vinyl plastic or standard 16" x 18" mesh metal window screen fashioned to fit within the rim of a standard 6" one gallon, plastic nursery pot (Fig. 1). Foodplant was propagated in the pots. The cylinders were 12 to 18 inches tall to contain the foodplants. The cages were used both for mating and subsequent rearing of larvae.

The second were "tent" cages, consisting of 0.75 inch white PVC tubing joined with standard fittings to form approximately 4 foot square by 3 foot tall frameworks which were covered with flexible plastic window screen. The tops, or roofs, were affixed using Velcro strips to facilitate access to the cage interiors. The tent corner posts were driven into the ground and the bottom edge of the screens buried to prevent loss of adults (Fig. 2). The tent cages were placed over closely planted clumps or individual large foodplant specimens in the field. Tops were necessary because rain can collect on exposed screens to create drops large enough to drown adults in cages. This was necessitated because of water pooling on the screen top with concentration and drowning of adults present.

Maintenance of adult viability in the gallon cages depended on regular feeding of a 20% honey in water solution once daily for about one hour. Earlier *ad libitum* feeding led to bloat and early death. Tent cages were placed over large foodplants with abundant flowers, which provided a sufficient natural nectar source.

Larvae were usually removed from both cage types at various stages as discussed below. These larvae were maintained individually in one ounce polystyrene cups (creamers) using either foodplant pieces or artificial diet. Pupation took place in the cups.



Fig. 1



Fig. 2

- Fig. 1. Gallon cage for controlled rearing of Palos Verdes blue butterfly.  
 Fig. 2. Tent cage for naturalistic rearing environment for Palos Verdes blue butterfly.  
 Fig. 5. Screen cylinder to allow eclosion and wing expansion of captive butterflies.  
 Fig. 6. Failed wing expansion in Palos Verdes blue butterfly.  
 Fig. 7. Aberrant Palos Verdes blue butterfly with reduced underside secondary macules.  
 Fig. 8. Aberrant Palos Verdes blue butterfly with exaggerated postmedial underside macules.  
 Fig. 9. Palos Verdes blue butterfly larvae with distended prothoracic segments.  
 Fig. 10. Male Palos Verdes blue butterfly caught in spider web.



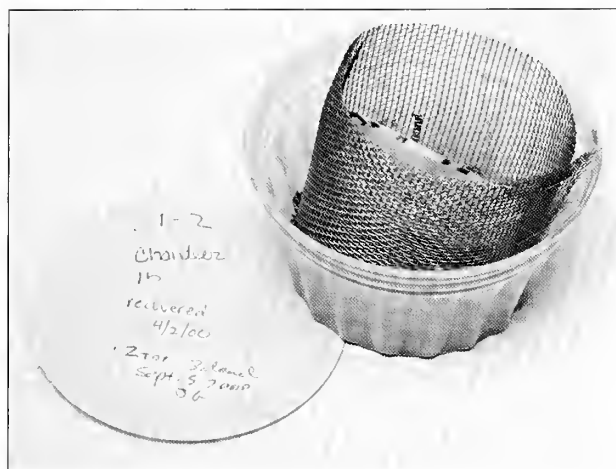


Fig. 5

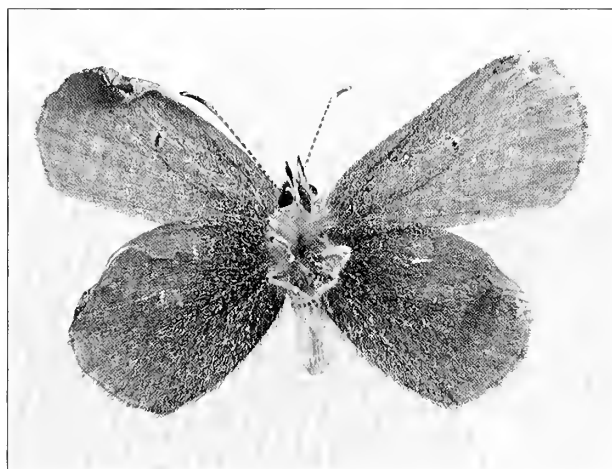


Fig. 6

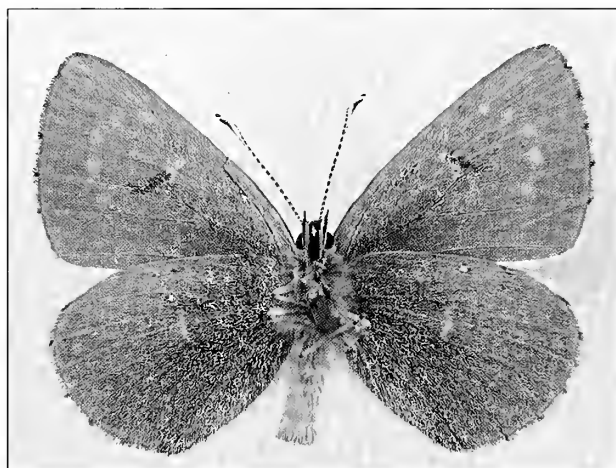


Fig. 7

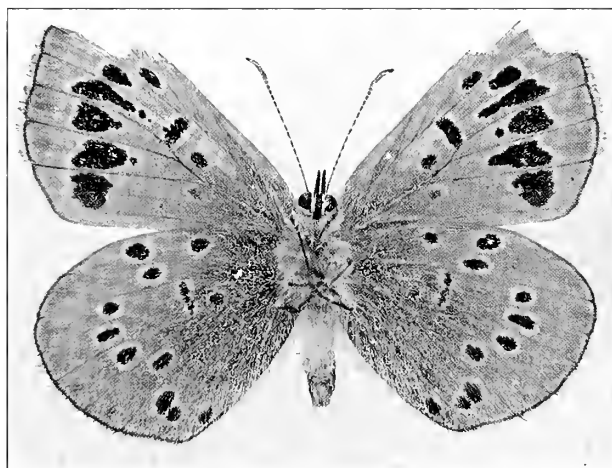


Fig. 8



Fig. 9

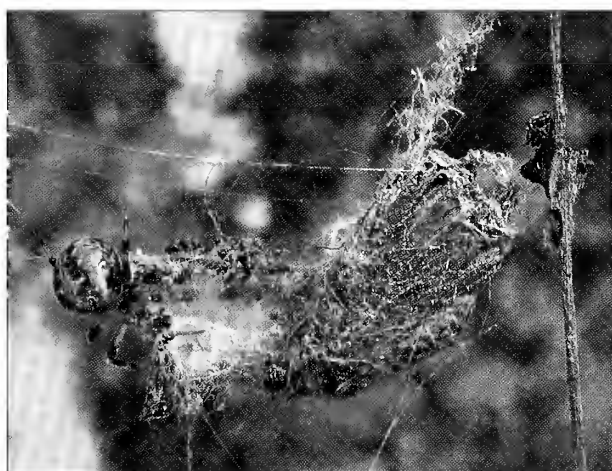


Fig. 10

## REARING METHODS AND LIFE HISTORY CHARACTERISTICS

### Pupae and Diapause

The pupae diapause under refrigeration and are synchronized for eclosion under continuous cold. We determined that eclosion occurs about two weeks following removal from cold to ambient temperature ( $\sim 20^\circ\text{C}$ ). In 2002 pupae were removed on February 24 and began eclosion March 8, the last adult emerging March 16. Time from removal from refrigeration to eclosion was normally distributed with a mean of 16 days (Fig. 3). Of 342 possible viable pupae from 2000 and 2001, 165 (48%) eclosed. Although many of the non-eclosed pupae were probably not viable, some fraction remained in diapause. Left under ambient conditions without refrigeration, eclosion can extend over a period of at least six weeks. Refrigeration is therefore a useful technique to synchronize eclosion to facilitate mating in the captive rearing setting.

Immediately following removal from refrigeration, all pupae were weighed to estimate how many were viable. A frequency distribution of all pupal weights after diapause showed a distinct bimodal distribution (Fig. 4), while pupae before diapause show a normal distribution (Longcore et al. 2002). After diapause, weights of pupae less than

50 mg formed a normal distribution (skewness = 0.19; mean weight =  $27.2 \pm 10.0$  s.d.), and weights of pupae greater than 50 mg formed a normal distribution (skewness =  $-0.10$ ; mean weight =  $86.1 \pm 17.2$  s.d.). Pupae less than 50 mg were assumed to be not viable, while pupae greater than 50 mg were assumed to be potentially viable. The hypothesis that 50 mg indicates a cutoff for viability was partially confirmed by the pattern of eclosion. No pupae below 50 mg produced adults, while 48% of those presumed viable did.

### Eclosion

Adults from fourteen pupae were unable to escape the pupal cases or failed to expand their wings. Most, if not all, were failures due to faulty physical environmental condition, e.g. positioning of the pupae that prevented their normally grasping a structure that would provide leverage to crawl from the pupal case. Although such failures must occur in nature, our artificial system is likely flawed. Fig. 5 illustrates the screen cylinders within which pupae are placed for eclosion. Emerging adults can climb the screen wall to allow full wing expansion. Fig. 6 illustrates an individual with failed wing expansion.

### Mating

Mating lycaenid butterflies in captivity has a variable success rate. Hoegh-Guldberg (1979)

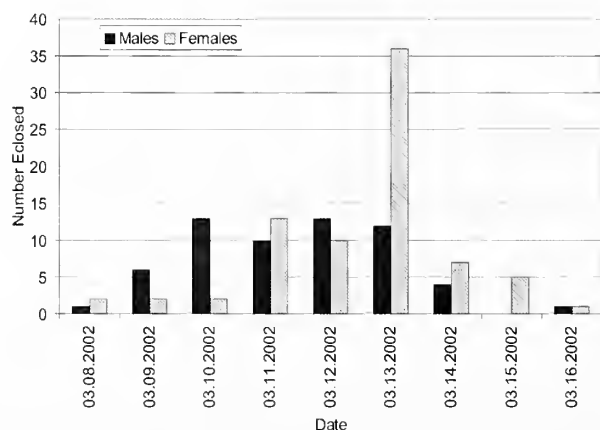


Fig. 3. Eclosion of adult Palos Verdes blue butterflies under laboratory conditions. Pupae were removed from refrigeration on February 24, 2002.

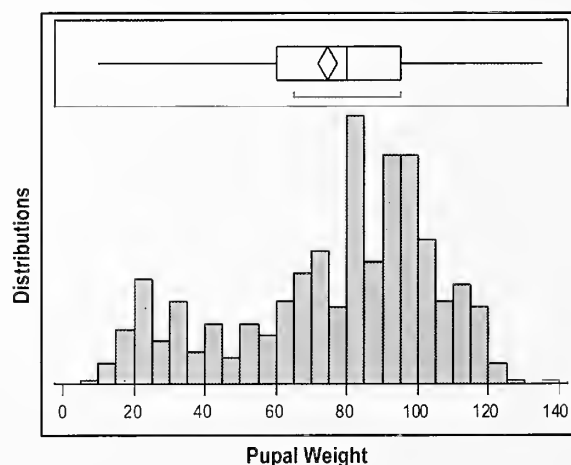


Fig. 4. Distribution of Palos Verdes blue butterfly pupal weights in mg when removed from refrigeration to break diapause. Note bimodal distribution with peaks at 90–100 mg and 20–30 mg (skewness =  $-0.64$ ).

successfully used very small plastic containers to mate European *Aricia* species, provided outdoor light was used combined with good ventilation. K. Shurian (pers. comm.) required hand manipulation to mate *Polyommatus* (*Argodiatis*) species. J. Thomas (pers. comm.) was finally successful inducing mating in *Maculinea* (considered congeneric with *Glaucopsyche* by some authors) when he released adults in outdoor walk-in screen houses. This last result prompted our design of the tent cages for year 2000.

The key factors to induce mating of Palos Verdes blue butterfly are temperatures of 18–25 °C under full sunlight. These factors critically impinge on cage design because high temperatures of insolation must be avoided. Ventilation thus becomes a factor, and care must be taken to maintain high humidity. A final factor for success is aging and feeding males for at least one day prior to mating attempts. Females are immediately competent to mate on eclosion, and we have an impression that females become increasingly reluctant to mate with age.

Although we had observed mating in the gallon cages in our earliest work from 1996, results were variable. The construction of tent cages in 2000 provided an apparent ideal environment for mating. After we fabricated two prototype units the mating problem was immediately solved. When the first set of adults was introduced, matings occurred

within minutes, a phenomenon we never noted in the gallon or other small cages.

#### Oviposition and the egg stage

Eggs are laid singly, usually on the foodplant flower buds and developing seedpods, secondarily on young stems and leaves. We have only limited data for average egg production per female because most rearing was performed using several mating pairs that were not individually segregated. From counts made, however, we observed a maximum of 187 eggs per female with many females yielding no eggs or a few sterile eggs. The latter cases were clearly the result of mating failure, a commonplace occurrence in our work with small cages. Sterile eggs were revealed by collapse of the egg between 5–8 days after laying. Eggs normally hatch in 8–10 days under ambient March temperatures.

During the 2002 breeding cycle, 14 “gallon” cages, each with two pair of adults, yielded none to about 60 eggs per cage, with a total of 317 eggs from 14 cages for an average of 11 per breeding pair (Table 1). The egg counts are approximate minimum values because of the difficulty in making accurate counts with the dense plant material present.

The Palos Verdes blue butterfly uses two foodplants on the Palos Verdes peninsula, deerweed (*Lotus scoparius*) and rattlepod (*Astragalus tricopodus*)

Table 1. Number of eggs laid, larvae and pupae recovered from 12 “gallon” cages each with two pairs of adults. Two foodplants employed.

Cage	foodplant	# eggs	larvae recovered	pupae
1	<i>Astragalus</i>	none		
2	<i>Astragalus</i>	none		
3	<i>Astragalus</i>	none		
4	<i>Astragalus</i>	40+	15	none
5	<i>Astragalus</i>	20+	10	none
6	<i>Lotus</i>	50+	25	3
7	<i>Astragalus</i>	60+	20	6
8	<i>Lotus</i>	40+	25	none
9	<i>Lotus</i>	50+	15	none
10	<i>Lotus</i>	50+	30	19
11	<i>Lotus</i>	none		
12	<i>Lotus</i>	none		
13	<i>Lotus</i>	3	2	1
14	<i>Astragalus</i>	4	4	2
Estimated total		317+	146	28

*lonchus*). Both were offered to the adults confined in the gallon cages (Table 1). There were no choice options in 2002, but egg productivity was similar on the two resources: 124 eggs on seven rattlepods and 193 eggs on seven deerweeds.

During earlier work we found egg counts per individual female varied widely from none (mating failure) to 187 per female. In 2000 we recovered a minimum of 500 eggs from 17 females in a walk-in screen house over an 18 day period.

### Larvae

There are four larval instars. The first are translucent off-white color, cylindrical, and bear many black, long setae. At this stage the larvae are very fragile and can only be moved using fine camel hair brushes or "Q-tips." In the laboratory these neonates often move from their birthplace, leave the foodplant (even if it is robust and healthy appearing), and are subject to loss. The reason for moving is unclear; while neonates occasionally leave apparently healthy plants, they almost always leave plants with aphid infestations.

Among larvae, neonate/first instar loss was high in gallon cages kept in the laboratory. Aside from the losses associated with plant condition (aphids, wilt), a test was made in year 1999. A set of 44 fertile eggs was transferred to cups with a few fresh flower buds (deerweed) just prior to hatching. All hatched, but only 20% (9/44) survived to second instar. In a parallel trial, 35 fertile eggs were transferred to buds placed on artificial diet. All neonates perished by drowning, either by wandering onto the wet diet surface or from condensate. To what extent handling itself was responsible is not known, but the effect of handling is likely not trivial. This experiment ruled out the use of artificial diet for neonate larvae, and confirmed the observation of high mortality during the first instar.

Later (second and third) instars were usually lost to apparent disease, but at a far lower rate. During 2002, disease losses were from a microsporidium and possibly a virus, discussed below. Fewer than a dozen larvae were lost over the years showing symptoms of Bt. Last instar larvae were rarely lost, with fungal infection being the the most common cause.

In our earliest attempts at rearing the necessity of individual confinement was implemented to avoid cannibalism. Initially third instar larvae were isolated in cups and fed pieces of foodplant. Because

it was necessary to replace the material daily, we switched to artificial diet for these later instar larvae.

### Artificial diet

The diet designed for larval growth is given in the appendix. Rearing on artificial diet in individual containers eliminates cannibalism, provides broad-spectrum antibiotics that virtually eliminate bacterial infection, and prevents losses from predation and parasitoids. Diet feeding was not without problems; including fungal growth on frass and, during 2002, refusal of many individuals to feed. The first necessitated frass removal every few days. The second necessitated adding fresh foodplant to the containers.

We would use the diets for early instars, but in addition to neonate drowning, second instars usually refused to feed. We found that when transferring second instars on foodplant pieces (such as a flower bud), the larvae would not accept the diet until achieving at least late third instar.

The reason that most of the 2002 larval population refused the diet is unclear. Diet with the same components had been almost universally accepted before 2002. Oddly, the green hairstreak (*Callophrys affinis perplexa*) controls we reared in parallel did feed on diet that they had in all earlier trials refused. We cannot explain the phenomenon and did not have the time to experiment.

The use of artificial diet in individual containers provided antibiotic and antifungal compounds that likely had a salutary effect. Green fungal growths were mostly confined to frass, never on the surface of the diet. When frass was removed, no residual fungal growth occurred.

However, fungal growths were a problem on some of the larvae themselves. These infections were associated with the ninth segment honey-gland and were usually fatal. Fungal growths were associated with high humidity in the capped containers (the small air holes we punched did not significantly reduce container humidity) and the secretion of honeydew by some individuals.

Most larvae recovered from the tent cages were attended by Argentine ants (*Linepithema humile*). Indeed the presence of ants was our visual clue to locate larvae at low density in the tents. When transferred into cups, the ants usually were introduced with the larvae. Because of their strong fidelity this was a byproduct of the transfer. For

larvae with attendant ants, no fungal growths were recorded, which we attributed to the continuous removal of honeydew by the ants.

### Pupation

Pupation in nature takes place in the loose duff and micro-crevasses at the base of foodplants. On *Astragalus* plants, pupation sometimes occurs within seedpods. A loose girdle of a few silk threads binds the pupa to substrate. Less than 1% failure of pupation has been observed (none in 2002), usually because the larval skin could not completely shed.

During the 48-hour prepupal transition the larva is immobile while preparing for ecdysis. This is a period of extreme vulnerability to attack by predators (see below). However, under laboratory conditions predators are excluded.

Following pupation, specimens are left to harden for a week. Then the pupae are removed from cups, cleaned by washing in a 5% tween 80 solution, dipped in a 10% bleach solution, washed in distilled water, and placed on a tissue paper pad in a clean cup. After one to two months the cups are placed into plastic shoeboxes over a layer of sterile pumice stone. The boxes are placed into a refrigerator at ~4 °C. The pumice is soaked with distilled water monthly to maintain humidity.

The 2002 pupae were weighed in August with the lightest weighing 60 mg. Because all pupae have a certain likelihood of remaining in diapause, all will be set out for rearing during the 2003 season. Multiple year diapause is a common strategy of insects in unpredictable climates (Scott 1986), and Palos Verdes blue butterfly is no exception.

## PROBLEMS ENCOUNTERED

### Aberrant Adults

Two classes of morphological anomalies were observed. The first involved defective legs. Four males and three females (4.1%) had truncated or missing tarsi. Because butterfly legs are rarely inspected either in collections, and even less often in nature, comparison of frequency of these defects to natural populations is not possible.

There were two wing pattern aberrations: 1) greatly reduced (N=11) or absent (N=17) underside secondary macules (Fig. 7) and 2) exaggerated postmedial underside macules (N= 3, Fig. 8).

Frequency of both aberrant forms is very low in nature and collections across all populations of the species. It is noteworthy that both leg anomalies and wing aberrations were significantly clustered in the set of adults emerging during the first three days of eclosion ( $15/32 = 0.47$  versus  $12/135 = 0.09$ ). We believe this is indicative of some thermal shock associated with premature ontological stages in some pupae when they were removed from refrigeration. Although there are no supporting data, the pattern implies the anomalies were developmental and not genetic.

### Diseases

Lepidoptera are susceptible to a wide variety of infective diseases (Boucias & Pendland 2001). Most knowledge of diseases is a consequence of economic importance for potential specific pest control. Demographics are unquestionably affected by all disease organisms that in turn may have profound impacts on density dependent population regulation and adaptive processes. Disease organisms may indeed account for some of the order of magnitude differences periodically seen in densities of adjacent populations of species where no visible resource variation is apparent. Although not tested, the hypothesis is plausible and offers one explanation of why Palos Verdes blue butterflies are sparse by comparison with nearby southern blue butterfly populations (*Glaucopsyche lygdamus australis*).

The high density monoculture of captive breeding programs provides a high risk environment for disease. Disease control is a key management factor, with all categories of infective agents likely to play a role. Bacteria, virus, fungi, nematodes, and microsporidia have all affected lepidoptera breeding programs (Tanada & Kaya 1993).

The sporogenic bacterium *Bacillus thuringiensis* (Bt) has proven a potent and widespread pathogen. Although observed in prior years, during 2002 we found no larval death from apparent Bt infection. Symptoms are cessation of feeding followed by sudden eversion of the hind gut through the rectum and almost immediate death. Bt symptoms and etiology are well known. The bacterium is apparently ubiquitous with a variety of genotypes occurring in nature with variable infectivity (Tanada & Kaya 1993). Under natural conditions epizootics are uncommon, but do occur in confined breeding.

Bt infestations may become endemic in breeding colonies with sublethal infections common. The use of antibiotics in defined diets usually maintains control, although the possibility of resistant strains arising is always present. Many other potential bacterial pathogens are likely as well.

All four major groups of viruses are known lepidopterous pathogens. Most virus usually cause rapid death in larvae terminating with a very characteristic "wilt" (Hunter-Fujita et al. 1998). Recently, Reoviruses (CPV) have been shown with serious chronic effects on insect breeding as they can be maternally transmitted (Hunter-Fujita et al. 1998). We have never observed apparent losses from classic "wilt" disintegration.

We experienced an unusual infection in a few second and third instar larvae exhibiting completely distended prothoracic segments (Fig. 9). The affected individuals ceased eating. This etiology was only seen in 2002. Of 28 noted, 20 died and 8 recovered to continue normal development. The symptom appeared only with plant-fed individuals, none with diet-raised stocks. The disease is noteworthy because some recovery occurred. The causal agent was probably a microsporidian (see Boucias & Pendland 2001). Microsporidian *Nosema* species have become endemic in pink bollworm laboratory stock. These are difficult to control, can contribute to reduced fertility in females, and are transovarian transmitted.

Besides the fungal infection noted on the larval honey gland, entomophagous fungi known from other lepidoptera have potential deleterious effects. We have not detected these. Nematodes constitute the last major potential pathogens. We have no evidence of nematode presence in our stock.

Practical control of all the above pathogens relies on cleanliness and frequent disinfection in the laboratory and use of biocide chemicals. Thus the use of defined diet, which is virtually sterile and contains antibiotics and fungicides, offers some protection in high density cultures. The apparent freedom of pathogenicity during the outdoor tent breeding suggests possible protection as a result of low density in an open complex ecosystem.

### Parasitoids

Although potential parasitoids that attack all life stages are doubtless present, none have been found during any facet of the breeding program. Given

exposure in the tent cages, it is particularly surprising that trichogrammid wasps have not been recovered from eggs. In spite of the tents providing access to most potential parasitoids, none have been observed.

### Predators

Two predators have killed individuals in the tent cages. Several species of spiders construct webs in the tents that have trapped adults (Fig. 10). Others likely prey on larvae, although direct attack has never been observed. Small larvae do disappear. Care must taken to remove all spiders from the laboratory, where predation by spiders is possible. We have also documented predation by yellowjackets on adult butterflies (Lipman et al. 1999), but this predator is adequately excluded by the tent and gallon cages.

The most serious predation has been from the abundant European earwig, *Forficula auricularia*. The earwig is one of the most common ground dwelling insects on the site. The 2001 rearing program was devastated by earwigs as a consequence of permitting larvae to pupate in the tent cages. Table 2 gives the results of egg production, observed larvae, pupae recovered and approximate earwig density from the cages. The earwig problem was unrealized until after pupa recovery when the significant correlation became clear. The density of earwigs better explained the number of pupae recovered on the ground in the cage than any other factor. Later tests, placing mature (hardened) pupae with earwigs did not result in predation. We hypothesize that heavy predation took place during the 48-hour period of prepupal quiescence when the new pupa exoskeleton is thin.

### Costs

Breeding results from 1995 to 1998 were poor, but increased effort yielded 627 pupae in 1999, 968 in 2000, with a setback to 299 in 2001 and 168 in 2002. The captive breeding program has improved and unlimited captive rearing seems attainable, given no unforeseen consequences. In 1999 the costs in time and material was about \$15,000, a cost per pupa (625) of \$25. The costs were about the same in 2000, with \$25,000 required to produce about 1,000 pupae. In both 2001 and 2002 costs

Table 2. Pupa recovery from the nine tent cages used for captive propagation of the Palos Verdes blue butterfly at DFSP, 2001. Number of breeding adults placed in cage, foodplant species enclosed, cage bottom, estimated egg and larval density, pupae recovered, and numbers of earwigs (*Forficula auricularia*) noted at time of recovery.

Cage No.	No. Adults*	Food Plant	Ground Cover	Oviposition Dates/ N	Larvae N & notes	Pupae N	<i>Forficula</i>
1	77	L	GC	3/17-4/20 >>100	>>100 noted	6, base of FP	>100
2	71	L	GC	3/17-4/20 ca 100	ca 100 noted	5, base of FP	>100
3	61	A	B	3/17-3/25	Few late instars Aphid defoliated	12, base w 12 in A. pods	>100
4	62	L	GC	3/17-4/5 few	Few early & late instars	none	>>100 highest
5	83	L	B	3/20-4/10 >300	Many, 40 late instars 4/15	24	~100
6	92	L	B	4/1-4/17 >300	Many all instars	108	6
7	81	L	B	3/21-4/15 >100	Many all instars	68	>25
8	66	A	B	3/21-4/1 few	None seen, aphid defol.	5, w 2 in>50 A. pods	
9	80	L	GC	3/21-4/12 >>100	Few noted after 4/15	71	>25

\*25 were variously lost during handling

Foodplant: L = *Lotus scoparius*, A = *Astragalus tricopodus*

Ground Cover: B = bare, GC = ground cloth of 4 mil black plastic

increased to about \$50 and \$100 per pupa respectively. Given that commercial rearing of lepidoptera for biological control and butterfly house display programs is on the order of \$0.07 to \$3.00 (depending on size and quantity), there is ample room to reduce costs. However, it must be recognized that the Palos Verdes blue butterfly is a diapausing species so high labor is required for short periods. This life history constraint does not provide for an efficient economy of scale. Lastly, both the facility and methodology are not yet optimal.

## CONCLUSIONS

Observations to date provide insights that may be conveniently considered as key factor analyses under laboratory conditions. Both fecundity (number of eggs produced per female) and fertility (frequency of fertile eggs) varied enormously. Although few individual females were scored, fecundity varied from none (mostly copulation

failure) to nearly 200 eggs. Fertility was usually 100%, discounting those females (see Table 1) who laid only a few sterile (collapsed) eggs or no eggs at all. The cause of infertility is assumed a failure of mating. Whether mating failure was intrinsic (genetic) or environmental is not known, although the latter is highly likely given the general mating success always noted in tent cages.

We conclude the following:

1. In comparison with three other lycaenid butterflies we have reared (Mattoni 1988), the Palos Verdes blue butterfly has been the most difficult.

2. Mating and rearing can be conducted effectively in outdoor tent cages. When late larval instar larvae are seen, they should be transferred to small cups on diet. This combined approach has so far provided the best results for mass rearing.

3. For special cases where small (e.g., gallon) cages are used, females should be permitted to oviposit for short periods of no more than 2–3 days. The cages should then be placed open, or partially



screened, in a protected outdoor location to avoid aphid infestation. Larvae should be permitted to mature within the enclosed cages. When near pupation, the larvae should be transferred to individual cups.

4. No evidence of parasitoid impact was found, even using the outdoor tent cages. Disease may or may not be an issue. The key factor limiting productivity appears to be providing an optimal environment for foodplant maintenance.

5. A pupation medium should be developed to permit ecdysis without loss to predators (earwigs) in both tents and small cages. Efforts to date indicate earwig predation is the major cause of loss if larvae are allowed to pupate outdoors.

6. Field collected females and males should be introduced into the breeding system to minimize loss of rare alleles.

7. Genetic studies would be appropriate to determine the extent of inbreeding both in the wild and captive populations. Comparative adult densities in nearby southern blue populations indicate that foodplant density is not limiting for the Palos Verdes blue butterfly.

8. Emphasis of the program must be on the capability of producing large numbers of offspring.

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## APPENDIX: LYCAENID SYNTHETIC DIET (MATTONI)

200 g dried green lentils  
800 ml distilled water

Place in one liter stainless beaker.  
Bring to boil and leave one hour.

Add in order:  
9 g bacto-agar  
25 g wheat germ  
5 g bacto yeast extract  
5 g Wesson salt mix  
10 g cellulose flour (Solka floc or equiv.)  
5 g sucrose  
2 g ascorbic acid  
2 g potassium sorbate  
2 g methyl paraben  
0.8 choline chloride  
0.25 g B-sitosterol  
0.25 g chlortetracycline  
0.25 g 50% procaine penicillin  
0.4 ml linseed oil (raw)

Heat mixture in boiling water bath (or double boiler on hotplate) until temperature reaches 85–90 °C, stirring occasionally.

Place mixture directly over low heat (flame or electric element) stirring constantly until mixture just comes to boil (necessary to dissolve agar).

Cool to about 80 °C.

CAREFULLY pour about 1/3 into (Waring) blender, blend for few seconds, after initial splashing, continue pouring remainder into blender until all well blended (about 30 seconds).

Dispense into containers (we use automatic pipette to dispense 5 ml aliquots into 1 oz creamers set in trays for easy handling), immediately cover with clean paper towels. Refrigerate when set (about 15 minutes), tightly enclosing trays in clean plastic bags. Can be stored under refrigeration for 60+ days.

### Alternatives

1. May substitute baby lima beans or other beans for lentils.
2. May substitute complete defined vitamin mixes, or multivitamin tablets for yeast extract.



## Note

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# Records of the host plants and clutch sizes of *Acraea* butterflies (Lepidoptera: Nymphalidae)

## INTRODUCTION

The study of butterfly-host plant relationships has been central to our understanding of evolution since Ehrlich and Raven's (1964) seminal paper on coevolution. The advent of molecular phylogenetics has recently permitted many of Ehrlich and Raven's original hypotheses to be rigorously tested (Janz & Nylin 1998). However, incomplete host plant records and the difficulty of judging the veracity of many reported host associations have hampered all these investigations.

The genus *Acraea* (Lepidoptera: Nymphalidae) is a potentially interesting group in which to investigate host-plant relationships for two reasons. First, the genus very speciose, containing over 240 species, and therefore provides many potential data points. Second, these species feed on a diverse range of hosts covering at least 24 different plant families (Ackery et al. 1995). In this paper we both confirm existing host records reviewed by (Ackery et al. 1995) and report new ones.

## METHODS

Egg batches or larvae were collected from Mabira forest and Kampala, both of which are in southern Uganda. Larvae were initially reared in petri dishes and then transferred to two litre jars. Approximately 30 larvae were reared together and each morning the jars were cleaned and the larvae fed fresh shoots. Compared to other butterfly species, *Acraea* caterpillars were not particularly susceptible to disease.

Adults are easily mated in small hanging cages (30-90 cm diameter) and will lay a batch of eggs the subsequent day if they are placed in a jar containing the food plant in the dappled shade.

## RESULTS

The host plant records are given in Table 1. The host plant of *A. vivianna* was previously unknown. We have also recorded a new host family for *A. encedon* and a new host genus for *A. quirinalis*. In addition, two always species were reared on British plants, although they had higher mortality than on their natural hosts. *Acraea eponina* was reared on *Tilia cordata* Mill. (Tiliaceae; small leaved lime) and *A. encedon* on *Urtica dioica* Linnaeus (Urticaceae; stinging nettles).

*A. vivianna* and *A. eponina* both feed on *Triumfetta rhomboidea*. However, these butterflies occur in very different habitats, nine groups of eggs or caterpillars collected from forest margins and clearings were all *A. vivianna* while 98% (n=99) of those from open country were *A. eponina*. The early stages of *A. vivianna* have not previously been recorded, the eggs, larvae and pupae were, on casual examination, indistinguishable those of *A. eponina*.

*A. encedon* was recorded on *Desmodium salicifolium*, the host plant of the closely related butterfly *A. encedana*. The eggs and larvae of *A. encedana* were always much commoner on this plant than those of *A. encedon*, even when adults of the later species appeared to be the most abundant.

*A. acerata* is a major crop pest of sweet potatoes (*Ipomoea batatas*), a staple food in Uganda. Crop damage was found to be especially severe in dryer places, where the caterpillars may virtually defoliate entire gardens.

## DISCUSSION

These results suggest that our knowledge of the host associations of *Acraea* butterflies is far from complete. Five of these species of caterpillar were collected without knowledge of the identity of either the plant or the butterfly. One of these five records

proved to be new host families and a second was a new genus. This is all the more remarkable given that this sample is strongly biased towards the commoner species of *Acraea*.

One record of particular interest is that *A. encedon* feeds on both the legume *Desmodium salicifolium* and the monocotyledon *Commelina benghalensis*, and in the lab it would also feed on the stinging nettle *Urtica urens*. This butterfly is closely related to three less common species (Pierre 1981), each of which feeds on one of these host genera; *A. encedana* feeds on *Desmodium salicifolium*, *A. encoda* Pierre 1981 on *Commelina* and *A. necoda* Hewitson 1861 on *Urtica* (and some other plants).

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**Table 1.** Host plant records, \* marks records which are either a new host genus or a new family (i.e. records not included in (Ackery, et al. 1995). The clutch sizes given are those of egg batches collected in the wild, with the number of clutches in brackets.

Butterfly	Species	Host-plant	Family	Mean clutch size	Description of how eggs are laid
<i>A. acerata</i> Hewitson 1874	<i>Ipomoea batatas</i> Linnaeus		Convolvulaceae	165 (n=20)	Single layer, touching
<i>A. bonasai</i> Sharpe 1890	<i>Triumfetta macrophylla</i> Schum		Tiliaceae	198 (n=3)	Single layer, spaced out
<i>A. ancedana</i> Pierre 1976	<i>Desmodium salicifolium</i> Poir		Fabaceae	106 (n=4)	2-3 layers, touching
<i>A. ancedon</i> Linnaeus 1758	* <i>Desmodium salicifolium</i> Poir		*Fabaceae		Single layer, touching
	<i>Commelina benghalensis</i> Linnaeus				
<i>A. eponina</i> Cramer 1780	<i>Triumfetta rhomboidea</i> Jacq.		Tiliaceae	122 (n=43)	Single layer, spaced out
<i>A. quirinalis</i> Grose-Smith 1900	* <i>Laportea ovalifolia</i> Chew		Urticaceae	51 (n=13)	Single layer, spaced out
<i>A. vivianna</i> Staudinger 1896	* <i>Triumfetta rhomboidea</i> Jacq.		*Tiliaceae	162 (n=3)	Single layer, spaced out
<i>A. zetes</i> Linnaeus 1758	<i>Barteria acuminata</i> spp. <i>fisculosa</i> Baker		Passifloraceae		

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## Opinion

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# Notes on the evolution of unpalatability in butterflies by means of individual selection

In the following note, arguments are presented which challenge: 1) the evolution of unpalatability in butterflies by means of individual selection, 2) the hypothesis suggesting that Batesian mimics would have a good opportunity to evolve unpalatability by individual selection, and 3) an existing hypothesis for a mechanism of transition from Batesian mimicry to Müllerian mimicry.

R. A. Fisher (1930) recognized that natural selection acting on individuals was a plausible force leading to evolution of distastefulness. The arguments for the evolution of unpalatability by means of individual selection are essentially as follows: An individual carrying a mutation that renders it less palatable, and results in it being rejected more often by predators, will have a selective advantage over normal individuals similarly attacked if the more distasteful insect is able to escape and reproduce more often than the "normals" (Benson 1971; Harvey & Paxton 1981). The fundamental prerequisite for evolution of unpalatability in butterflies, by means of individual selection, requires that the distasteful mutant of a palatable species must survive the attacks of predators and subsequently reproduce.

Because there is no visual difference between the distasteful mutant and the normal palatable form, the only way for a bird (the main vertebrate predator of butterflies) to perceive that the mutant is distasteful is by tasting it. To survive, the mutant must be rejected by the predator and released unharmed after being caught, tasted and found distasteful. To be efficient and effective at this task, the specific chemical compound(s) that render the mutant unpalatable cannot be a toxin. This is because of the manifestation of the symptoms of toxicity are not instantaneous. For example, a bird vomits after consumption of a monarch (*Danaus plexippus* L.) containing at least one ED<sub>50</sub> cardiac glycosides or several monarchs with low toxicity containing a total of one ED<sub>50</sub> of the glycosides (Brower & Fink 1885). To be eaten, the monarch

must obviously be acceptable by the predator as palatable regardless of any toxin it contains. Logically, the chemical factor causing unpalatability must possess a flavor that the bird predator is able to taste, and on the basis of this taste, is then able to reject the prey promptly without harming it.

To taste a butterfly and release it unharmed (without disrupting the integument), the factor(s) rendering the butterfly distasteful (unpalatable) must be located on the water-impermeable outer surface of the wing or outer surface of the chitinous body and must be water-soluble. Only water-soluble substances in form of free molecules can be tasted (Zweers 1982). The outer surface of the chitinous integument and the wings, however, does not contain, nor it can retain, water-soluble molecules (see Kassarov 1999).

The only way a bird could taste a butterfly and release it unharmed requires tasting without disrupting the integrity of the integument. This may happen only with the minimal loss of a small part of the wing caught in the beak - beak-mark tasting, or by non-lethal pecking. Such precision is beyond the ability of a bird's gustatory apparatus. The apparatus is simply not sensitive enough in terms of the very small number of taste receptors (taste buds) and the manner of their distribution on the tongue and in the beak cavity. The issue is discussed in detail in Kassarov (1999, 2001)

By deduction the unpalatable mutant individual gains no protection from predator attack because there is no way for the birds to differentiate visually between the normal form and the new noxious mutant. To achieve protection, it is essential that the noxious mutant advertises its distasteful quality, i.e., it must acquire an aposematic color pattern as the theory of aposematism postulates. Thus, to avoid being attacked, the mutant must differ from the palatable normal form not only by taste. There must be a visible difference that the bird is able to discriminate and perceive as a warning signal that

the mutant individual should be avoided. However, the last condition further necessitates previous encounter(s) of the bird predator with the mutant, memorizing the encounter, recognizing the mutant and then differentiating it from the normal form. The mutant has to survive the encounter(s) to become fixed as a new form.

Thus, it seems reasonable that development of an aposematic (advertising) color pattern necessitates a simultaneous mutation in the color pattern of the mutant. However, novel warning variants gain no protective advantage from their color pattern, since predators cannot have previously encountered and learned their color patterns. This leads to frequency-dependent disadvantage of a rare variant within a species (Mallet & Singer 1987). Also, warningly colored variants may be more conspicuous than non-aposematic prey. "At very low frequencies, a conspicuous mutant will not be remembered however memorable it is because predators nearly always encounter it only once" (Mallet & Singer 1987). And "So little information is retained about conspicuous mutants by predators that their conspicuousness is a constant detriment because it increases the rate at which they are detected" (Servedio 2000). To perceive the new conspicuous color form (the mutant) as conspicuous, the bird has to taste it without disrupting the integrity of the integument and release it without diminishing its future reproductive success. The whole story gets entangled in a vicious circle.

Another factor widely considered responsible for the survival of aposematic butterflies (insects) is toughness and resilience of the integument. Wiklund and Järvi (1982) suggested that because many aposematic species are tough and difficult to kill (Cott 1940; Edmunds 1974), toughness would reduce the risk of lethal attack and allow enough distasteful individuals to escape to favor distastefulness. But "this begs the question of how toughness evolves" (Endler 1991). Thus, toughness of the integument cannot be considered as protecting the distasteful mutant of a palatable butterfly because the evolution of toughness must precede the mutation, or both must appear simultaneously or be genetically linked. There are no published data concerning a causal relationship between toughness of the integument and chemical compounds that may render the insect distasteful. Such a relationship could exist if based on a chemical reaction as, for

example, polymerization, that provides a hardened chitinous integument. It seems highly improbable that chemical compounds that supposedly render a butterfly distasteful simultaneously cause the integument to become tough and resilient.

Thus, a survival of the mutant must be stochastic: the mutant simply was not caught by a predator and managed to mate and reproduce by chance. Random predation, given a very low initial frequency, may confer relative protection of the rare mutant that might thus increase its chance for survival. The mutant may subsequently increase in frequency, but this will occur only as long as the frequency of the mutant remains very low (see Mallet & Singer 1987, Endler 1991).

While the unpalatable mutant remains very rare, it will be effectively "hidden". So, what selective forces could lead to an increase in frequency of the mutant? There is no apostatic selection because the bird predator cannot exert a selective pressure. In order to exert selective pressure, the bird must be able to recognize both the unpalatable mutant and the palatable form and avoid the mutant. Since there is no visual difference between the distasteful mutant and the palatable form, the frequencies of mutants and palatable normals in the population will not be subjected to selection, and the rare mutants will receive no advantage.

Huheey (1961) stated that "it seems likely that unpalatable characteristics are often eliminated from populations when in the incipient stages simply because the bearers did not survive the tasting procedure and the characteristic was not as yet sufficiently widespread to benefit the entire population;" and further suggested, "that Batesian mimics would have a good opportunity to evolve unpalatability by individual selection. Being protected from attack by the model, any tendency to develop distasteful qualities will be enhanced since predators would attack and taste them cautiously." Under this scenario, it is the palatable mimic that mutates to unpalatability. Since both mimic and distasteful mutant are protected by the model, and because there is no visual difference between the mimic and the mutant, both would be attacked with equal caution. The bird perceives them as the same variety in the same manner as a distasteful mutant of a palatable butterfly not mimicking a distasteful one. Since the mutant is, in fact, a mimic of the model, but unpalatable (distasteful), and the bird perceives it as

a mimic, this will lead to an increase of the frequency of the distasteful form in the population. As a result, there will be a new distasteful form that is phenotypically unrecognizable from the mimic and with a color pattern more or less resembling the model (depending on how advanced is the progression of the Batesian mimicry). The more perfect the mimic, the more closely the mutant will resemble the model. Accordingly, the community will maintain both the distasteful Batesian model and the distasteful mutant differing from the model not only by strength of distastefulness but also by the substance(s) determining the distastefulness.

Huheey (1961) also advanced a mechanism for the transition from Batesian to Müllerian mimicry. The problem with this mechanism is that again, not only is the distasteful mutant protected by the model, but the palatable Batesian mimic is protected as well. The model equally protects both. However, because there is no visual difference between the Batesian mimic and the mutant, the mutant is not protected by apostatic selection and bird predation cannot be a selective factor for the stabilization of the mutant.

Evolution of Müllerian mimicry via Batesian mimicry predicts that the palatable Batesian mimic should be selectively eliminated from the population by predation: i.e. it must lose protection by the model. The mutant, however, should remain fully protected by the model and continue to reproduce. Accordingly, the community will maintain both the distasteful Batesian model and the distasteful mutant. I cannot perceive a mechanism by which the bird predator can both selectively eliminate the mimic and selectively protect the rare mutant. Thus I consider the hypothesis suggested by Huheey (1961) questionable. On the basis of the arguments presented, I question the proposed mechanism for the development of unpalatability in palatable butterflies based on individual selection.

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